cancer treatment reports

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Proceedings of the 16th Annual Meeting
of the Society for Economic Botany:
"Plants and Cancer"

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cancer treatment reports

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The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
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All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements (published bimonthly) which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

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Fellowship in Medical Oncology
Third Annual Meeting of the Association of Community Cancer Centers

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Proceedings of the 16th Annual Meeting of the Society for Economic Botany: "Plants and Cancer"

University of Maryland, Baltimore
June 15–18, 1975

Editors: Robert E. Perdue, Jr
Jonathan L. Hartwell

The Society for Economic Botany is an association of individuals interested in the past, present, and future uses of plants for man.

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Introduction

Ralph J. McCracken[1,*]

My colleagues in the Agricultural Research Service (ARS), US Department of Agriculture (USDA), join me in expressing appreciation for the opportunity to participate in this series of discussions on the potentials and problems in applying natural plant products to a major human health problem. This symposium on Plants and Cancer offers us the opportunity to learn more about each other's work and objectives so the research programs can be made even more complementary and mutually reinforcing.

Within the USDA there is strong support from other agencies in carrying out the plant exploration and procurement program, especially from the Foreign Agricultural Service (FAS), the Animal and Plant Health Inspection Service (APHIS), and the Forest Service (FS). FAS personnel are especially helpful in the overseas exploration and procurement phases, APHIS in expediting the entry of plant materials through plant quarantine, and FS in obtaining cooperation from forest rangers and others during the domestic procurement phases of our program. Cooperation between other federal departments and private agencies has been excellent. Many contracts with and grants to individuals, hospitals, industrial organizations, universities, and other establishments have been made by the National Cancer Institute (NCI) to achieve the goals of the cancer chemotherapy program.

I should like to discuss the first part of the symposium title—"Plants"—since that is obviously the area in which the ARS can contribute through its long history of research on useful plants. We have heard much lately about the use of plants, and the products derived from them, in meeting world food problems. But of equal concern and importance is human health and in this respect plants have much to contribute that is of therapeutic value. Unfortunately, this aspect of man's use of plants hasn't received as much recent attention, either in terms of publicity or research priority, as has the "food and feed" use of plants. It is therefore an advantageous time to present this symposium in order to bring this aspect into perspective.

As pointed out in a recent National Research Council report, "Productive Agriculture and a Quality Environment," there are thousands of complex molecules synthesized by plants, both higher and lower, which are not known to biochemists. Some of these are highly toxic and could become hazardous to the environment. They don't accumulate in the environment because microbes utilize and degrade them, but toxicity is a biologic activity and where there is biologic activity we must search for compounds of therapeutic value. The enormity and magnitude of this task can be appreciated when it is realized that there are 230,000 species of higher plants and an even greater number of microorganisms. Each functions as a rather unique chemical factory—synthesizing complex compounds. Approximately 25,000 species of higher plants have been screened to date for anticancer activity. More than 2000 of these showed sufficient activity to merit further evaluation.

The question may be asked, why is the USDA interested and why is the ARS conducting the plant procurement phase of the cancer program? Since its establishment in 1862, the USDA has been interested in identifying useful plants. The USDA has a long and proud tradition of plant exploration and introduction, originally developed and continually enlarged because of the paucity of useful crop plants in North America. No major crop plant is indigenous to the US. The Office of Foreign Seed and Plant Introduction was set up in the USDA in 1898 as a focal point for plant exploration and introduction. Medicinal aspects of this program were given special emphasis in 1949 when a major program in the department was organized to explore for plants to be screened for precursors of cortisone. Within a few years a program to search for natural plant products useful in the treatment of hypertension was added. These were cooperative programs with the National Institutes of Health. Shortly thereafter, the present ARS was established, with a Division of Plant Exploration and Introduction, later to be known as the New Crops Research Branch. This group developed an extensive program for procurement of plant materials to be screened as

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*_Reprint requests to:_ Dr. Ralph J. McCracken, Agricultural Research Service, US Department of Agriculture, Washington, DC 20250.

sources of chemically unique seed oils and of fibers suitable for the manufacture of paper.

Thus, the ARS had available a valuable resource in plant exploration, introduction, and procurement when the NCI initiated the plant screening program in 1960. With the reorganization of the ARS in 1972, procurement of plants for biologic and chemical screening was centered in its newly established Medicinal Plant Resources Laboratory at the Beltsville Agricultural Research Center.

The ARS is pleased and proud to be a participant with the NCI in this useful activity and to participate in this symposium on Plants and Cancer. The agency plans to continue its plant procurement program as long as needed. We feel there is a real future for natural products research.

History of the National Cancer Institute and the Plant Screening Program

Saul A. Schepartz[1,*]

This is a brief outline of the history of the NCI, its drug development program, and specifically, the plant product program of the NCI. It is not possible in this paper to do justice to these programs, and those who are interested in a more complete review and analysis may refer to the review of the Cancer Chemotherapy National Service Center (CCNSC) program (1) and the comprehensive history of the Chemotherapy Program (2).

The NCI was established in 1937. Its mission was "to provide for, foster and aid in coordinating research relating to cancer." It might be of interest to note that the NCI appropriation for fiscal year 1938 was $400,000. As the first and largest of the institutes of the NIH, the NCI has often pioneered in the development of new approaches and types of programs in attempting to pursue the problems for which it is responsible. During those early years, studies on plant extracts were carried out by Dr. M. J. Shear, Laboratory of Chemical Pharmacology, NCI. Dr. Jonathan L. Hartwell, also reporting in this symposium (3), was active in that program; his studies resulted in the isolation of podophyllotoxin, the new analog demethylpodophyllotoxin, and the α- and β-peltatins. Under Dr. Shear's direction, this drug development program was the first attempt to develop an inter-institutional cooperative chemotherapy program. Unfortunately, because of the lack of an appropriate mechanism for carrying out clinical studies, it was not possible to continue this organized effort.

During the early 1950s, a great deal of interest was generated in the scientific and lay community on the need for an expanded capability for the screening of new agents and other aspects of chemotherapy. At that time, the Sloan-Kettering Institute (New York, NY) was the only major resource available for the primary screening of new agents and it was agreed that a program should be established within the NCI that could be made available to chemists and others interested in having their drugs evaluated. This program was originally envisioned as a voluntary and cooperative arrangement rather than a so-called directed program which would finance an expanded research grants program with a central information exchange and a small resources program under contract for providing chemicals and screening services to grantees. It appears that the scientific community clearly did not wish to establish a targeted, directed program. It is not clear if Congress had the same viewpoint. However, in April of 1955, the CCNSC was established within the NCI to coordinate this first national voluntary cooperative cancer chemotherapy program. The initial organization was actually under the joint sponsorship of the NCI, American Cancer Society, Damon Runyon Fund, Atomic Energy Commission, Veterans' Administration, and Food and Drug Administration. The original objectives included the procurement of drugs, screening, and other necessary preclinical studies (supported by contracts), and the clinical evaluation of active drugs through special grant support. All of these functions were clearly "service" in nature. By 1958 however (only 3 years later), the program had evolved into a largely industrial-type, targeted drug development program. This had occurred because of pressure from academic sources submitting compounds, massive participation of the pharmaceutical industry, increasing demands for new drugs by the cooperative clinical groups, and probably most important, continuing Congressional pressure through increased appropriations each year. Although the philosophy and mission of the CCNSC had changed from a "service" program to an integrated research and development program, it was > 10 years before the name was changed to reflect this philosophic change. Today we have the DR&DP as a major component of the total DCT program, conducting various projects oriented toward the development of new and better methods of cancer treatment involving all modes of therapy.

Returning to the early years of the CCNSC, the input of synthetic compounds for screening began early in 1956 and was followed shortly thereafter by a large number of fermentation products. The input of plant extracts for screening did not start on a systematic basis until 1961 although a significant

[1]Drug Research and Development Program (DR&DP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

*_Reprint requests to:_ Dr. Saul A. Schepartz, DCT, NCI, Bg 31, Rm 3A51, National Institutes of Health (NIH), Bethesda, Md 20014.

TABLE 1.—Plant extracts submitted for screening

Yr submitted	No. of plant extracts submitted
1956–1958	~ 500
1959	~ 1000
1960–1961	~ 5500
1962	5200
1963	5200
1964	4000
1965	3816
1966	3620
1967	6253
1968	4245
1969	5109
1970	3499
1971	3015
1972	10,434
1973	11,661
1974	5830

number of plant materials were screened prior to that time. Table 1 presents a summary of the number of plant extracts submitted to the NCI for screening since the beginning of the program. The input of plant extracts during the first few years primarily represented materials obtained from the US Department of Agriculture (USDA) that had been used originally for earlier programs[2] at their Eastern Regional Laboratory, as well as plants from a variety of other sources. In July 1960, the first contract agreement was established with the USDA for the specific collection of new plants and it is after that period that the collection and acquisition of materials for screening became systematized. You will note a rather constant input of new plant materials from 1962 until 1971. During 1972 and 1973, the input was greatly accelerated in conjunction with the overall expansion of the program resulting from the enactment of the National Cancer Act of 1971. By 1974, it was realized that we could not maintain this level of input in relation to other parts of the program; it was therefore decided to again decrease the input to about 5000 materials per year.

The establishment of the program with the USDA in 1960 and the overall comprehensive effort in the development of plant materials occurred for specific reasons. By that time, the activity of the *Vinca* alkaloids had become known, and it was well established that plant materials had the potential of providing us with a tremendous variety of new structures, some of which should be useful in the treatment of cancer patients. The review of the CCNSC program, which was carried out in 1959, specifically recommended that plants be explored more extensively as a source of agents with antitumor activity. The program was developed in 1960 as a direct result of this recommendation, which I believe was an exceedingly wise one.

In addition to the program with the USDA, a program for the collection and preparation of extracts was also initiated in 1960 with the University of Arizona (Tucson). It was not until 1961 however, that the initial contract involving the isolation of active agents was initiated with the Research Triangle Institute (Research Triangle Park, NC) under the direction of Dr. Monroe Wall. Since that time, a number of contracts for the isolation of active drugs have been established and now represent a substantial ongoing program. Table 2 summarizes the funds that have been obligated to this area of the total NCI program from fiscal year 1961 to the present time. I should point out that these funds do not include resources devoted to the initial screening work carried out on the crude extracts or the bioassay of materials in the course of fractionation. This biologic testing activity represents a very substantial investment which is, of course, necessary for the successful development of new agents. In general, it can be noted from these figures that the program has grown significantly over the years with the exception of the period from 1968 to 1971. The decrease during that period reflects the general belt tightening that occurred in addition to the termination of a rather large drug development contract which included plant materials as well as other types of natural products.

The following is a brief outline of the manner in which the overall program operates. Some of this is discussed in considerably greater detail in other papers in this issue, but an initial overview might be

TABLE 2.—Funds obligated for isolation of plant products

Yr of obligation	Funds obligated ($)
1961	173,000
1962	194,000
1963	326,000
1964	379,000
1965	524,000
1966	575,000
1967	892,000
1968	814,000
1969	609,000
1970	558,000
1971	555,000
1972	745,000
1973	978,000
1974	945,000

[2]These extracts had been prepared primarily for screening for cortisone precursors.

worthwhile. Dried plant materials are obtained largely from the USDA as well as from the University of Hawaii (Honolulu) and from a number of additional smaller sources. These materials are sent to our contract facility at the WARF Institute (Madison, Wis) where extracts are prepared and concentrated. The extracts are then tested in any one of several screening laboratories utilizing both in vivo and in vitro experimental systems. Plants whose extracts demonstrate reproducible activity in one of these experimental systems are re-collected in larger quantities and retested. If the new collection still contains an active material, the plant is then assigned to one of several contract facilities for the chemical isolation of the active component or components. Laboratories currently carrying out this activity are shown in table 3. A new extract prepared by the chemist is tested. If this extract is active, the chemist proceeds with fractionation to isolate the active agent(s). The specific systems used for initial testing and for the bioassay of fractions have changed over the years. With the aid of the testing and bioassay systems, fractionation proceeds until a pure material is obtained. If that material meets our criteria for activity, uniqueness, etc, it is approved for development toward clinical trial. These development efforts include large-scale collection and production, synthesis (if necessary), formulation development, and large animal toxicology studies.

A number of materials have come through the program which are currently or have previously been in clinical trial. Because of the long time period involved in development, isolation, etc, I believe we are only now reaching the point of maximum return in terms of materials being prepared for development toward clinical trial. Several agents discussed in this issue hold high promise, I believe, as potentially useful drugs and I feel very optimistic that overall, the plant program will provide a valuable source for clinically active antitumor agents in the future.

TABLE 3.—Contractors isolating plant products*

Institution	Principal investigator
University of Arizona	Jack R. Cole
Arizona State University	George R. Pettit
University of Illinois	Norman R. Farnsworth
Research Triangle Institute	Monroe E. Wall
University of Virginia	S. Morris Kupchan

*As of June 1975.

REFERENCES

1. SESSOMS SM, COGHILL RD, and WAALKES TP. Review of the Cancer Chemotherapy National Service Center program. Cancer Chemother Rep 7:25–46, 1960.
2. ZUBROD CG, SCHEPARTZ S, LEITER J, ET AL. The Chemotherapy Program of the National Cancer Institute: history, analysis, and plans. Cancer Chemother Rep 50:349–540, 1966.
3. HARTWELL JL. Types of anticancer agents isolated from plants. Cancer Treat Rep 60:1031–1067, 1976.

Magnification of Plant Parts:

Habit: ♀ matured inflorescence and leaf x ½
top left: larger leaflet and part of rachis x ½
portion of branch showing spines x ½

♂ inflorescence - part of pyramidal panicle x ½
♂ flower x 10
♀ flower x 10
fruit x 3
seeds x 3

Fagara macrophylla (Oliv.) Engl.—The source of nitidine, this is a large forest tree of tropical West and East Africa. The active agent is concentrated in the rootbark. The rootbark from West Africa is a much richer source of nitidine than that from East Africa. The bark collected as a source of nitidine is taken from lateral roots growing near the soil surface. The tree has a taproot—specimens 4 inches in diameter or larger are not adversely affected by destruction of their lateral roots.

Plant Folklore: A Tool for Predicting Sources of Antitumor Activity?[1]

Richard W. Spjut and Robert E. Perdue, Jr[2,*]

SUMMARY

The National Cancer Institute's record of "active plants" (extracts which showed a significant inhibitory effect in experimental tumor systems) was compared with plants reported in folklore to have medicinal or poisonous properties. The occurrence of active plants was found to be higher in plants reported in folk literature than in plants collected at random, suggesting a correlation between plants used in folklore and those with anticancer activity.

[Cancer Treat Rep 60:979–985, 1976]

The Agricultural Research Service inaugurated a major procurement program in 1960 to supply plant samples for the Cancer Chemotherapy National Service Center, National Cancer Institute (NCI). The program was initiated to identify novel chemical structures produced by plants that might be useful in cancer treatment.

Procurement of plant samples was largely at random, with two simple guidelines: (*a*) any plant part or combination of plant parts would be acceptable if the sample could be supplied in an amount of 1 pound or more; and (*b*) duplication of species would be acceptable if samples were collected in different seasons or from different geographic areas.

A major collection of samples for this screening program was assembled in Ethiopia in 1961–1962. Extracts representing 43 species showed significant antitumor activity in laboratory animals or in cell culture. One species, *Argemone mexicana* L., had been collected because of a report that it was used in Africa and elsewhere for treating cancer; a second species, *Gloriosa simplex* L., had been collected because a native had volunteered that he used the tubers to treat gonorrhea.

A second field program in Ethiopia was conducted by Perdue in 1964–1965 to procure larger quantities of plant material for isolation and characterization of the chemical compounds responsible for the antitumor activity. At every opportunity the open-air markets were explored to gain information about local use of plants. Several plant products representing species sought as potential sources of anticancer drugs were found in these markets: bark of *Croton macrostachyus* Hochst. ex Delile, seed of *Embelia schimperi* Vatke and *Myrsine africana* L., and inflorescences of *Hagenia abyssinica* (Bruce) J. F. Gmel. All were used locally as anthelmintics, the preferred remedy being inflorescences of "koso" (*H. abyssinica*).

One plant with confirmed antitumor activity, *Cardiospermum halicacabum* L., could not be found in Ethiopia in adequate quantity. Soon after the field project was completed, a letter enclosing a few fragments of this plant was received from a Burmese volunteer. It was reportedly used in Burma for treating cancer and was said to be a common weed in rice paddies.

Two chemical compounds now of prime interest as potential new antitumor agents are from plants that have been widely used in Africa for a variety of maladies, including worms and cancerous tumors: bruceantin from *Brucea antidysenterica* J. F. Mill. and maytansine from several *Maytenus* species. There are two reports of the use of *Maytenus* species for treating cancer in Africa. One could have been prompted by newspaper reports about *Maytenus* in Africa (1). The second, however, is a valid folk remedy. A *Maytenus* species was identified as part of a mixture of plant material used for this purpose in South Africa.[3] This remedy was in use before

[1]Supported by agreement Y01-CM-40001.

[2]Medicinal Plant Resources Laboratory (MPRL), Plant Genetics and Germplasm Institute, Beltsville Agricultural Research Center, Agricultural Research Service (ARS), US Department of Agriculture (USDA), Md.

[*]*Reprint requests to:* Dr. Robert E. Perdue, Jr, MPRL, ARS, USDA, Beltsville, Md 20705.

[3]Harington JS. Personal communication to JH Hartwell, Drug Research and Development Program, Division of Cancer Treatment, NCI, Oct 28, 1969.

there was public knowledge of interest in *Maytenus* as the source of a potentially useful antitumor agent.

These and other observations (2,3) seem more than mere coincidence and have led to the question, "Can folklore be used as a tool in predicting sources of antitumor agents?" The objective of this paper is to answer this question, not to exhaustively explore the folk usage of plants.

DEFINITIONS AND SCOPE

The NCI has developed a screen of bioassays that is selective for chemical substances which might be useful for treating human cancer. In the preliminary screening, extracts which produce a significant inhibitory effect against tumor growth in laboratory animals or cell culture are designated as active (4). An active species is one represented by one or more active extracts; an active genus is one which includes one or more active species.

Active species include those with significant antitumor activity against test systems such as Adenocarcinoma 755, Sarcoma 180, and Walker 256 carcinosarcoma (WA). These test systems are no longer used in screening because it has become evident that they are not sufficiently predictive for clinical activity. Also, they are sensitive to tannins, phytosterols, and saponins, chemical agents almost ubiquitous among higher plants and which are not regarded as potentially useful antitumor agents (3,5). Nevertheless, it would be inappropriate to rule out WA, for example, because it is sensitive to tannins. While tannins are not regarded as being potentially useful new drugs, this is, at least in part, because they are not stable and the bioassay results are inconsistent. Tannins also are consistently highly toxic with low therapeutic indices, but this is also true of many other active agents identified by the screen.

In addition, these test systems, especially WA, are also sensitive to other types of compounds such as alkaloids which are potentially useful drugs. An example is thalicarpine (from *Thalictrum dasycarpum* Fisch. & Mey. & Ave-Lall.), which is active against WA and is now under clinical evaluation. Also, an appreciable number of species active originally only against WA have since been active against P388 lymphocytic leukemia suggesting that their activity is due to constituents other than tannins, phytosterols, or saponins.

Since the plant screening program began in 1960, the screen has changed, gradually but completely (4). To review the vast records of this program in order to limit this evaluation to plants tested against tumor systems now employed would be impractical.

The term folklore is used here broadly. It encompasses all reports implying that a plant produces a physiologic effect against any animal organism and includes plants used for medicinal purposes, insecticides, fish or arrow poisons, and those suspected or known to be poisonous to man or livestock.

Six compendia (6–11) were used to represent a broad spectrum of plants in folklore. These were selected because: (*a*) they deal primarily with plants from areas of the world where plants have been collected for antitumor screening, (*b*) Latin names of plants in these compendia are reasonably compatible with current botanical nomenclature, and (*c*) texts and/or indices were organized so that they were adaptable to use for our purpose. The compendia (6–11) and much of the other literature cited in this paper were reviewed to compile lists of plants used as anthelmintics and fish and arrow poisons. Botanical names were accepted as cited in the compendia; however, when lists of plants used for specific purposes were compiled, the Latin names were reviewed to eliminate synonymy and to assure that names used are those now generally accepted.

A list from the series of articles entitled Plants Used Against Cancer (2) was used as a basis of comparison between active genera and species and this one application in the folklore.

A list of active species identified by the NCI screen was compared with indices and texts of the compendia to gain a general overview of any possible correlation between plants with antitumor activity and those from folklore. This list of active species was compared with the list from Plants Used Against Cancer and the compilations referred to above to determine if there is any apparent correlation between antitumor activity and more specific uses.

THE "RANDOM" SCREEN: A BASIS FOR COMPARISON

Procurement of plant samples for primary screening has been focused on selected geographic areas. No two areas have been sampled equally and plants of many areas are not represented. Some species have been sampled one time, others two or more times. Plants have been selected largely at random, although some encountered have not been sampled because they were too small or uncommon to make their collection practical. The orchids, for example, are not well represented because they are usually not sufficiently common. As screening progressed, it became evident that the grasses and a few other

families were poor sources of activity and further procurement of these plants was discontinued.

In addition, it also became evident that certain families, such as the Apocynaceae, Celastraceae, Rutaceae, Simaroubaceae, and Thymelaeaceae, are good sources of activity and a special effort was made to seek out plants in these families for screening.

Finally, some suppliers of plants or extracts for screening have concentrated on medicinal plants. Thus, while the overall procurement of plants for screening has been at random, there are these notable exceptions.

Extracts representing 20,525 species in 4716 genera and 317 families have been screened for antitumor activity. Of those screened, 2127 species (10.4%) in 1225 genera (26%) are active. Because of the large number of genera and species screened, the percent of active species and genera provide a basis against which data compiled from folklore can be compared to determine whether it is a useful tool in predicting sources of antitumor activity in higher plants.

Many variables make it impossible to evaluate folklore by standard methods of statistical analysis. On the other hand, statistics can be compiled from folklore reflecting different points of view and compared with the yield of active genera and species in the random screen. Any one comparison will be without statistical validity. However, if these comparisons consistently show, or fail to suggest, a correlation between antitumor activity and folklore, valid conclusions can be drawn.

PLANT TAXONOMY AND ITS ROLE IN EVALUATION OF FOLKLORE

Taxonomists evaluate characteristics of organisms and categorize these by taxa that fall into a systematic hierarchy (species, genera, families, etc) in accordance with established rules of nomenclature. At the base of this classification is the species concept. Taxonomists often disagree on their interpretations of species, or interpretations may change with time as new evidence is found. For example, maytansine was isolated from an Ethiopian plant initially identified as *Maytenus ovata* (Wall. ex Wight and Arn.) Loes. under a conservative concept which recognized *M. ovata* as including plants from southern India and Africa. More recently, this genus was studied in detail for treatment in the new *Flora Zambesiaca* (12). The Ethiopian plant is now recognized as *M. obscura* (A. Rich.) Cufodontis. *M. ovata* is regarded as endemic to southern India. A species of *Maytenus* in Kenya and Tanzania, also formerly identified as *M. ovata*, is currently identified as *M. buchananii* (Loes.) R. Wilczek, and is now recognized as a superior source of maytansine.

Differing taxonomic concepts create difficulties in evaluating anticancer/folklore relationships. An example is a black-eyed susan that is reported to have been used in American Indian medicine under the name *Rudbeckia divergens* T. V. Moore (10). This name is acceptable under a very narrow species concept which considers minor morphologic differences as a basis for different species. Under a more conservative view, the plant is a variety of *Rudbeckia hirta* L. (13), an active species.

Generic concepts also differ. *Gymnosporia obscura* A. Rich. is reported to be used as a remedy for malaria in North and East Africa and *G. senegalensis* Lam. for several medicinal purposes elsewhere in Africa (14). Under current concepts of genera in the Celastraceae, these are both recognized as species of *Maytenus*. Thus, it is evident that when reports of folk use are recorded under names applied under one concept and active species or genera are identified under another concept, relationships between folklore and activity are easily overlooked.

Botanical nomenclature also creates difficulties. Many examples could be cited where a single species has been collected from different locations and described by two or more botanists as two or more species. This was especially true during the early period of geographic exploration, a period when scientific communication was inadequate. European botanists received vast collections of plants from expeditions to distant areas and independently two or more applied new and different names to the same species.

Many species are morphologic continuums with extreme morphologic variants, especially when they are distributed over a large geographic area. Many examples can be cited where the extremes came first to the attention of botanists and were described as two or more species.

While much has been accomplished, especially during the present century, in resolving taxonomic and nomenclatural problems by monographic studies of genera and by studies of regional floras, a great amount of information about folk use of plants is recorded under plant names that are outdated and any thorough evaluation of folklore must take this into consideration.

A genus usually includes more than one species. Important relationships can be overlooked when one species with a folk use is closely related to another that is active. An example is the genus *Thalictrum*, which includes about 150 species. *Thalictrum polycarpum* (Torr.) Wats. is an active species; *T. fendleri* Engelm. ex Gray is used medicinally by

Indian tribes of Nevada (8). Their recognition as separate species is questionable (15).

At the other extreme is *Carex*, a taxonomically complex genus including possibly 2000 species (15). Uphof (16) cites one species, *Carex arenaria* L., used in medicinal folklore. Only one species, *C. occidentalis* L. H. Bailey, is active. These species belong to different sections of the genus suggesting they are not closely related. Furthermore, considering the size of this genus, it is probable that at least one species will be used medicinally in folklore and one species will show anticancer activity. Thus, the anticancer/folklore relationship involving *C. arenaria* and *C. occidentalis* may mean very little.

Plant taxonomy thus plays a very important role in comparisons of antitumor activity with folklore. In this paper, comparisons are made at both the genus and species level. At the species level, relationships between antitumor activity and folklore are likely to be closer than they appear because there are probably many examples where a plant with a folk use is closely related to an active species or where, because of synonymy, the folk use is reported under one name and the activity of the plant is recorded under another.

On the other hand, at the genus level, relationships between antitumor activity and folklore are probably not as close as they appear, for there will be genera-like *Carex* where the apparent relationship is probably without meaning.

GENERAL OVERVIEW

Six compendia dealing with medicinal and poisonous plants were reviewed to gain a general overview of relationships between antitumor activity and folklore. Table 1 shows the number of genera and species cited in each compendium and the number and percent active.

The percent of active genera cited in these compendia is consistently about double that of the random screen (26%). The percent of active species, with one exception, is 1.4–2.6 times greater than the yield of active species from the random screen.

Webb's guide (9) was reviewed to determine which plants are recorded as being poisonous (or suspected as being poisonous) and are also used for medicinal purposes. This compendium records 229 genera that include one or more species that are poisonous and one or more species that are used for medicinal purposes of which 153 (66.8%) are active. It records 196 species that are poisonous and used medicinally of which 37 (18.9%) are active. At the genus and species level, this suggests that poisonous plants used for medicinal purposes are more likely to be active than either medicinal *or* poisonous plants.

These comparisons individually have little, if any, validity. Collectively, however, they suggest there is a correlation between anticancer activity and folklore.

ANTITUMOR ACTIVITY OF PLANTS USED AS ANTHELMINTICS

The observation of an apparent relationship between anticancer activity and use of plants in Ethiopia as anthelmintics was one factor that prompted the question reflected by the title of this paper. A comprehensive but not exhaustive review of the literature (6,7,9,14,17–41) was made to determine which species are used as anthelmintics. Plants used as internal remedies by humans for helminth infestations such as tapeworm, roundworm, guinea worm, elephantiasis and shistosomiasis were recorded. After a review of the nomenclature to eliminate synonymy, 668 species in 457 genera and 128 families were represented. The number of genera and species screened totaled 433 and

TABLE 1.—No. of genera and species cited in 6 medicinal and poisonous plant compendia and No. and percent of genera and species with antitumor activity*

		Genera			Species		
Investigator(s)	Ref No.	No. listed	No. active	% active	No. listed	No. active	% active
Medicinal plants							
Krochmal and Krochmal	6	207	131	63.3	251	67	26.7
Quisumbing	7	580	271	46.7	855	140	16.4
Train et al	8	142	77	54.2	214	32	15.0
Webb	9	398	228	57.3	529	87	16.5
Weiner	10	285	156	54.7	388	73	18.8
Poisonous plants							
Kingsbury	11	282	159	56.4	488	105	21.5
Webb	9	433	211	48.7	760	70	9.2

*Not all of the genera and species listed have been screened for antitumor activity.

TABLE 2.—No. of genera and species in 4 selected families used against cancer (2) that have been screened for antitumor activity and the No. and percent of active genera and species

	Genera				Species			
	Recorded	Screened	No. active	% active	Recorded	Screened	No. active	% active
Family								
Fabaceae	87	84	42	50.0	180	136	25	18.4
Liliaceae	32	30	15	50.0	89	51	10	19.6
Rubiaceae	30	23	15	65.2	48	18	6	33.3
Rutaceae	8	8	4	50.0	22	21	4	19.0
Totals	157	145	76	52.4	339	226	45	19.9

482 respectively. Of these, 52.2% of the genera and 29.3% of the species are active. The percentage of active species is about three times greater than the random screen; thus, it appears that use of plants as anthelmintics is a good criterion for selection of plants for anticancer screening.

ANTITUMOR ACTIVITY OF PLANTS USED AGAINST CANCER

Hartwell (2) compiled a list of > 3000 species of plants reported in folklore for treating cancer and other conditions symptomatic of cancer such as warts and tumors. The higher plants included 2725 species representing 1201 genera and 185 families. Many of these have been screened for antitumor activity. Because of the large number of species involved, it was not practical to completely review the nomenclature of all of these plants to accurately identify those screened for antitumor activity. Four families were selected as generally representative of plants used for this purpose. They were the Fabaceae, Liliaceae, Rubiaceae, and Rutaceae. Table 2 shows the number of genera and species recorded by Hartwell, the number screened for antitumor activity, and the number and percent active. The number of genera and species screened were 145 and 226 respectively. Of these, 52.4% of the genera and 19.9% of the species are active. The percentage of active species is about twice as great as that identified by the random screen.

ANTITUMOR ACTIVITY OF POISONOUS PLANTS

A comprehensive but not exhaustive review of the literature (7,9,14,37,42–50) was made to compile lists of plants used as arrow and fish poisons. Botanical nomenclature was reviewed to eliminate synonymy.

Fish poisons.—Two hundred and fifty-four species were recorded representing 164 genera in 64 families. Screening has included 145 of these species and one or more species in 158 genera. Of these, 38.6% of the species and 65.8% of the genera are active.

Arrow poisons.—Seventy-six species were recorded representing 63 genera in 29 families. Screening has included 46 of these species and one or more species in 60 genera. Of these, 52.2% of the species and 75% of the genera are active. Plants used as arrow poisons seem to be a better source of activity than those used as fish poisons.

It is worthy of note that fish poisons often merely stun fish to bring them to the surface while arrow poisons are intended to kill. Also, arrow poisons are used on warm-blooded animals while fish poisons are used on cold-blooded animals.

THE FOLKLORE RECORD FOR PLANT SOURCES OF ANTITUMOR DRUGS

The most convincing answer to the question posed in the title of this paper is offered by the one agent of plant origin that is now a drug of choice in the treatment of cancer and by those plant products selected by the screening program that are now regarded as having the greatest promise as antitumor drugs.

One of the most useful drugs in the chemotherapy of acute childhood leukemia and some other cancers is vincristine,[4] from the periwinkle *Catharanthus roseus* (L.) G. Don. The initial discovery of the antileukemic activity of this drug resulted not from a search for antitumor activity, but was incidental to a search for compounds with hypoglycemic activity. The plant was under investigation in two different laboratories because of its folk use as a remedy for diabetes (52).

While most compendia deal with plants poisonous to man and livestock, one deals specifically with plants poisonous to man. Hardin and Arena (51) record 113 genera and 141 species as internal poisons—"those that cause a chemical or physiological

[4]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

disturbance or death, when eaten." Known fatalities are attributed to 42 of the species. These are plants native to or cultivated within the US. Screening for antitumor activity has included all of the genera and 129 of the species: 66.4% (75) of the genera and 50% (58) of the species are active.

Poisonous plants are clearly a good source of antitumor activity, especially those used as arrow poisons or reported as toxic to man.

DISCUSSION AND CONCLUSIONS

The data in the previous sections support the conclusion that folklore is a useful tool in predicting sources of antitumor activity. The closest relationship seen has been between antitumor activity and plants known to be poisonous. This suggests that future screening of plant extracts might be more profitable if greater emphasis is given to bioassays designed to detect toxicity. This supports the choice of the KB cell culture, a very sensitive and inexpensive screen for toxicity, used almost since the inception of the plant screening program.

The relationship between antitumor activity and plants used as anthelmintics compared with the relationship between antitumor activity and general medicinal use suggests that further comparisons could be useful if they are based on plants with toxicity to an infesting organism, but minimal toxicity to the human host.

Although folklore is a useful tool for predicting sources of antitumor activity, this does not mean that it should be used to guide screening. It is an additional tool, now recognized because of the massive data generated by the screening program. The random screen has revealed active plants not known to be used in folklore.

It is evident that, had antitumor screening been guided strictly by the knowledge of medicinal folklore and poisonous plants, the yield of active species would have been increased by a minimum of about 50%, probably by 100%. Screening guided by this knowledge might have been appropriate had the program been intended to be of short duration.

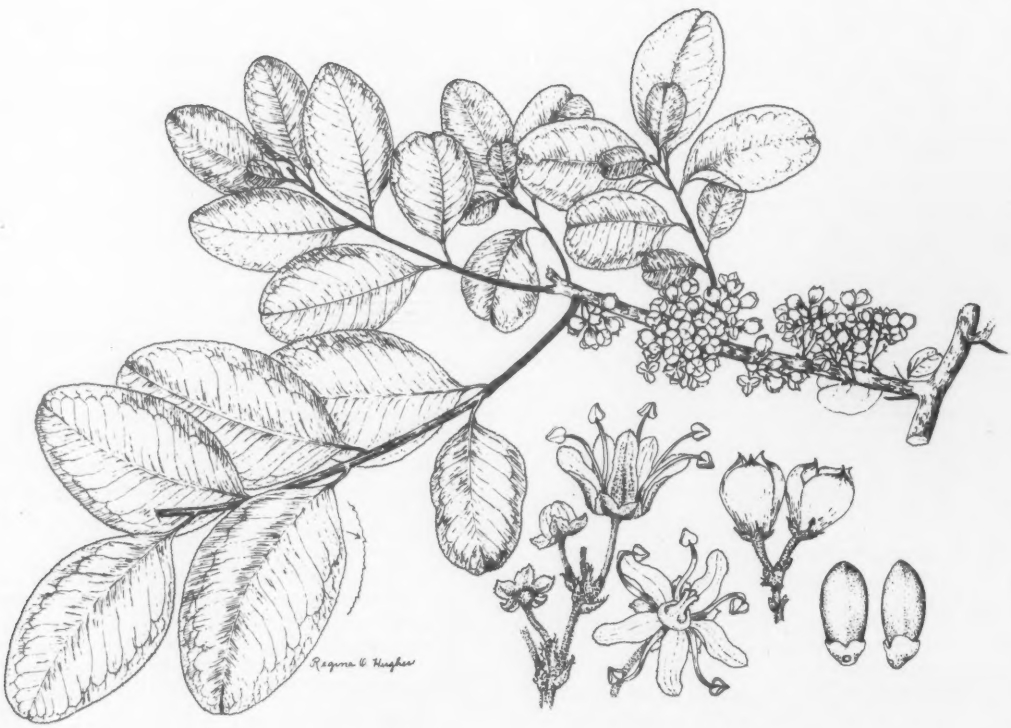
Under present NCI guidelines, the compounds most likely to be selected for preclinical evaluation are those active against the P388 and/or L1210 leukemias and the B16 and/or Lewis lung solid tumors (survival systems). Of the 21 most promising compounds listed in Hartwell's table 1 (3), eight compounds fit these criteria. These are bruceantin (from *Brucea antidysenterica*), 3-desmethylcolchicine (from *Colchicum speciosum* Stev.), ellipticine (from *Ochrosia moorei* [F. Muell.] F. Muell.), indicine *N*-oxide (from *Heliotropium indicum* L.), maytansine (from *Maytenus buchananii* and its relatives), taxol (from *Taxus brevifolia* Nutt.), tripotolide (from *Tripterygium wilfordii* Hook. fil.), and holacanthone (from *Holacantha emoryi* Gray). Seven of the eight genera from which these compounds were isolated are represented with species reported in folklore to be poisonous to man, animals, or insects (7,9,11,14,17,19,46,49,53,54). The one exception is *Holacantha emoryi* which is a monotypic genus of restricted distribution (northern Mexico, southern California, and Arizona) and no reference to folk use for this plant has been found. Of the seven remaining genera, all except *Tripterygium* and *Ochrosia* are also represented with species used in folklore for treating cancer (2). But, over the long term, random procurement has brought before the screen not only plants known to be used in folklore, but also others for which there is no folklore record but which have yielded useful antitumor agents.

Field botanists in underdeveloped areas where the local population is often largely dependent on herbal medicine should make an effort to determine what plants are used medicinally and which are poisonous and make a special effort to obtain samples of these for screening.

REFERENCES

1. KABUGUA M. I am not a witch doctor. *In* Nation Magazine. Nairobi, Kenya, July 28, 1972.
2. HARTWELL JL. Plants used against cancer. A survey. Lloydia 30:379–436, 1967, and ten additional installments ending with 34:386–437, 1971.
3. ———. Types of anticancer agents isolated from plants. Cancer Treat Rep 60:1031–1067, 1976.
4. ABBOTT BJ. Bioassay of plant extracts for anticancer activity. Cancer Treat Rep 60:1007–1010, 1976.
5. HARTWELL JL, and ABBOTT BJ. Antineoplastic principles in plants: recent developments in the field. *In* Advances in Pharmacology and Chemotherapy (Garattini S, Goldin A, Hawking F, ET AL, eds). New York, Academic Press, Inc, 1969, vol 7, pp 117–209.
6. KROCHMAL A, and KROCHMAL C. A Guide to the Medicinal Plants of the United States. New York, Quadrangle, The New York Times Book Co, 1973, 259 pp.
7. QUISUMBING E. Medicinal plants of the Philippines. Technical Bulletin No. 16. Philadelphia, Department of Agriculture and Natural Resources, Manila, 1951, 1234 pp.
8. TRAIN P, HENRICHS JR, and ARCHER WA. Medicinal Uses of Plants by Indian Tribes of Nevada: Contributions Toward a Flora of Nevada, No. 45. Revised edition, with summary of pharmacologic research by WA Archer. A series prepared through the cooperation of the National Arboretum and the Plant Introduction Section, ARS, USDA, Beltsville, Md, 1957, 139 pp.
9. WEBB LJ. Guide to the Medicinal and Poisonous Plants of Queensland. Bulletin No. 232. Council for Scientific and Industrial Research (Gourley JJ, government printer). Melbourne, Australia, 1948, 232 pp.
10. WEINER MA. Earth Medicine-Earth Foods (Plant Remedies, Drugs, and Natural Foods of the North American Indians). New York, Macmillan Publishing Co, Inc, 1972, 214 pp.
11. KINGSBURY JM. Poisonous Plants of the United States and Canada. Englewood Cliffs, NJ, Prentice-Hall, 1964, 626 pp.

12. ROBSON N. New and little known species from the flora Zambesiaca area. XVI. Taxonomic and nomenclatural notes on Celastraceae. Bol Soc Broteriana 39(ser 2):5–56, 1965.
13. PERDUE RE, JR. Synopsis of *Rudbeckia* subgenus *Rudbeckia*. Rhodora 59:294–298, 1957.
14. WATT JM, and BREYER-BRANDWIJK M. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Edinburgh, E. and S. Livingstone Ltd, 1962, 1456 pp.
15. MUNZ PA. A California Flora. Berkeley, University of California Press, 1965, 1681 pp (cf, p 107).
16. UPHOF JC. Dictionary of Economic Plants, 2nd ed. Lehre, Germany, Verlag Von J. Cramer, 1968, 591 pp.
17. Medicinal Plant Branch, Pakistan Forest Research Institute. A Note on the Plants of Medicinal Value Found in Pakistan. Abbottabad, Pakistan, 1956, 55 pp.
18. BOUQUET A, and DEBRAY M. Plantes Medicinales de la Cote D'Ivoire. Travaux et Documents de LORSTOM, No. 32. Paris, 1974, 232 pp.
19. BURKILL IH. A Dictionary of the Economic Products of the Malay Peninsula. London, 1935 (2 volumes).
20. CHOPRA RN, NAYAR SL, and CHOPRA IC. Glossary of Indian Medicinal Plants. New Delhi, Council of Scientific and Industrial Research, 1956, 330 pp.
21. DALZIEL JM. The Useful Plants of West Tropical Africa. London, Crown Agents for the Colonies, 1937, 612 pp.
22. DUKE JA. Ethnobotanical observations on the Choco Indians. Econ Bot 24:344–366, 1970.
23. ERNST A. Plants used medicinally at Caracas, Venezuela, South America, and their vernacular names. J Bot (London) 3:143–150, 277–284, 306–322, 1865.
24. GITHENS TS. Drug Plants of Africa. Philadelphia, University of Pennsylvania Press, 1949, 125 pp.
25. GLOVER PE, STEWART J, and GUYNNE MD. Masai and Kipsigis notes on East African plants. Part 1. Grazing, browse, animal associated and poisonous plants. East Afr Agric Forestry J 32:184–191, 1966.
26. ———. Masai and Kipsigis notes on East African plants. Part 2. Medicinal uses of plants. East Afr Agric Forestry J 32:200–207, 1966.
27. GUILLARMOD JA. Flora of Lesotho (Bausutoland). Germany, J. Cramer, 1971, pp 408–458.
28. HERMAN M. Herbs and Medicinal Flowers (translated by G Jackman), Editions Minerva SA, Geneva. West Germany, 1973, 127 pp.
29. HOOPER D, and FIELD H. Useful plants and drugs of Iran and Iraq. Field Mus Nat Hist (Botanical Series) 9:73–241, 1937.
30. HOOPER D. On Chinese medicine: drugs of Chinese pharmacies in Malaya. Gardens' Bull Straits Settlement 6:1–163, 1929.
31. KROCHMAL A, WALTERS RS, and DOUGHTY RM. A Guide to Medicinal Plants of Appalachia. USDA Forest Service Research Paper NE–138, 1971, 291 pp.
32. Lipp FL. Ethnobotany of the Chinantec Indians, Oaxaca, Mexico. Econ Bot 25:235–244, 1971.
33. MARTINEZ M. Las Plantas Medicinales de Mexico, 5th ed. Mexico, Ediciones Botas, 1969, 656 pp.
34. MORTON J. A survey of medicinal plants of Curacao. Econ Bot 22:87–102, 1968.
35. ———. Folk Remedies of the Low Country. Miami, E. A. Seemann Publishing Co, Inc, 1974, 176 pp.
36. NAGATA KM. Hawaiian medicinal plants. Econ Bot 25:245–254, 1971.
37. PALMER E, and PITMAN N. Trees of Southern Africa. Cape Town, A. A. Balkema, 1972, 2235 pp.
38. PITTIER H. Manual de las Plantas Usuales de Venezuela. Caracas, Litografia de Comerico, 1926, 458 pp.
39. TURNER N, and BELL M. The Ethnobotany of the Coast Salish Indians of Vancouver Island. Econ Bot 25:63–104, 25:335–339, 1971.
40. UHE G. Medicinal plants of Samoa. Econ Bot 28:1–30, 1974.
41. VOGEL VJ. American Indian Medicine. Oklahoma City, University of Oklahoma Press, 1970, 583 pp.
42. ALLEN PH. South American arrow poisons. Ann Mo Bot Gard 34:234–241, 1946.
43. COLLINS WF, WILD H, and HOPKINS J. Poisonous plants of the Marandellas district. Rhodesia Agric J 47:106–125, 1950.
44. GARDNER CA, and BENNETTS HW. The toxic plants of Western Australia. Perth, West Australian Newspaper, Periodicals Division, 1956, 253 pp.
45. HOWES FN. Fish Poison Plants. Bulletin of Miscellaneous Information. Kew, England, 1930, pp 129–152.
46. HURST E. The Poisonous Plants of New South Wales. Sydney, Australia, 1942, 498 pp.
47. MCFARLAND JW. Poisonous plants used for fishing. Yosemite Natl Notes 30:14–21, 1951.
48. MOLDENKE HN. Poisonous Plants of the World, 3rd ed. Yonkers, NY, 1951, 25 pp.
49. QUISUMBING E. Vegetable poisons of the Philippines. J Forestry 5:145–171, 1947.
50. ROBB GL. The ordeal poisons of Madagascar and Africa. Harvard Univ Bot Mus Leafl 17:265–316, 1957.
51. HARDIN JW, and ARENA JM. Human Poisoning from Native and Cultivated Plants, 2nd ed. Durham, NC, Duke University Press, 1974. 194 pp.
52. CARTER SK. Clinical evaluation of drugs from plants. Cancer Treat Rep 60:1141–1156, 1976.
53. HOEHNE FC. Plantas Substancias Vegetais Toxicas e Medicinais. Sao Paulo, Brazil, Departamento Botanico do Estado, 1939, 355 pp (cf, pp 132–135).
54. SWINGLE WT, HALLER HL, SIEGLER EH, ET AL. A Chinese insecticidal plant, *Tripterygium wilfordii* introduced into the United States. Science 93:60–61, 1941.



Magnification of Plant Parts:

Habit x ½
Flowers x 6 (left, natural position; right, spread open)
capsule x 2½
seeds x 4

Maytenus buchananii (Loes.) Wilczek.—The source of maytansine, this plant is a rambling twisted vine-like shrub, widely distributed in tropical Africa but apparently most common in the Shimba Hills and environs of southeastern Kenya near the coast of the Indian Ocean. It is known to the Digo tribe which inhabits this area as Mudziadzyh. Maytansine is concentrated in the stemwood. The plant is easily propagated from cuttings or seed and seems readily adaptable to cultivation in the tropics.

Procurement of Plant Materials for Antitumor Screening

Robert E. Perdue, Jr[1,*]

SUMMARY

The plant procurement program is described with emphasis on proper documentation of plant samples for screening, the role of botanical nomenclature in a procurement program, and selection of field areas and implementation of field programs to assure screening of a broad diversity of plant species, genera, and families.

[Cancer Treat Rep 60:987-998, 1976]

The US Department of Agriculture (USDA), organized as an independent agency in 1862, has a long tradition of plant exploration and introduction beginning in 1898 when the Office of Foreign Seed and Plant Introduction was established. Thus, its valuable expertise in plant exploration, introduction, and procurement was available when the National Cancer Institute initiated its plant screening program in 1960. From 1960 until mid-1972, procurement of plant material for screening was the responsibility of the New Crops Research Branch, Plant Science Research Division, Agricultural Research Service (ARS), USDA. With the reorganization of ARS on July 1, 1972, this procurement became the responsibility of the Medicinal Plant Resources Laboratory (MPRL), ARS, USDA.

Initially, procurement was largely accomplished by field projects in the US and Mexico. Later, the scope of the procurement program became international, with field programs supported by Public Law 480 funds in Pakistan, Korea, Spain, Yugoslavia, Turkey, Uruguay, and Israel, and field projects conducted by MPRL botanists in Colombia and Eastern Africa. When the screening program expanded in July 1971, the increase was met largely by contracts with suppliers in foreign countries, who are now supplying about 90% of the plant material obtained for the screening program. The network of suppliers is growing, so that almost any plant needed will be readily available.

Invaluable cooperation has been received from the USDA's Foreign Agricultural Service, represented by Agricultural Attachés in > 60 foreign countries who provide an important link between MPRL and field programs conducted by contractors and MPRL botanists abroad.

Another USDA agency, the Animal and Plant Health Inspection Service (APHIS), has made valuable contributions to the procurement program. APHIS is responsible for preventing the importation of plants, animals, soils, etc, that might be hazardous to American agriculture. Plant quarantine regulations permit the direct importation of most dried plant material by any research facility, subject to inspection at port-of-entry, and the importation of seed through an APHIS Inspection Station. Imported living vegetative plant material is closely controlled and usually must be propagated under quarantine.

In smaller countries where no Agricultural Attaché is assigned, program interests are well represented by economic, commercial, or consular officers, all representatives of the State Department.

American embassies abroad provide valuable logistic and fiscal service. Under this unique system, contractors deliver plant material directly to Agricultural Attachés or other embassy representatives and receive prompt payment. After export permits are obtained by the embassy, shipments are delivered to a commercial exporter, where they are crated, transferred to a port, and loaded aboard ship. The exporter then bills the embassy, which pays the costs and charges the MPRL account.

Within the US, ARS has facilities that can be used in support of the chemotherapy program. At the Plant Introduction Station at Glenn Dale, Mary-

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land, near both Washington and Beltsville, cuttings or seed of plants of special interest can be propagated under quarantine and either held in greenhouses or distributed to other stations in Georgia, Florida, or Puerto Rico for maintenance. Some plants needed in large quantity are rare in nature and must be cultivated. Many ARS facilities are available for large-scale production of tropical, subtropical, and temperate species.

The success of the plant screening program depends heavily on effective plant procurement. This has been achieved, in large measure, because of the cooperation of many agencies and individuals, here and abroad.

Objectives of a Procurement Program

The primary objective of a plant procurement program is to bring as much chemical diversity before the screen as possible. This will be achieved by botanical diversity, ie, maximum representation of the genera and families of higher plants. In planning a procurement program it is important to recognize that those plant samples which yield active extracts must be re-collected in larger volume for fractionation to isolate active agents. A select few will later be needed in massive amounts as sources of chemical compounds for preclinical and clinical evaluation.

To assure both high quality and economy, field areas must be carefully selected and field programs carefully planned. Although some have proposed focusing plant procurement on remote and exotic areas, there is an adequate diversity of plants in areas which are easily accessible.

An efficient screening program which is based on a well-planned design will produce samples of interesting plants *which can be resupplied* to followup antitumor activity identified by preliminary screening.

Documentation of Plants Sampled

Every plant sampled for a screening program must be documented by several sets of carefully prepared, pressed voucher specimens, with flowers and/or fruit, dried in folds of newsprint. Good specimens are required for reliable identification when duplicate collections must be obtained for isolating active agents. If necessary, when re-collections are made, voucher specimens can be taken into the field for comparison.

Field notes must be made describing the plant and its environment, location, and abundance. Each specimen must be numbered and the same number recorded in the field notes and on all samples documented by the specimens (fig 1).

The screening program generates thousands of specimens documenting plants sampled. Vouchers documenting active plants are sent to the National Arboretum Herbarium for mounting and are returned and incorporated into the MPRL working collection so they will be accessible for reference. Three years after samples are submitted and assays are complete, the remaining specimens are sent to the Arboretum for mounting and permanent filing. Duplicate specimens are distributed to other herbaria so they will be available if a voucher is misplaced. At WARF, Inc, as a further precaution against error, a few representative fragments of samples are removed, identified by accession number, and filed before processing. After screening, these fragments or "reference samples" of active plants are returned to MPRL for permanent filing. Before a botanist returns to the field, most reference samples can be compared with voucher specimens to verify identity and to assure re-collection of the proper plant. Reference samples of roots and bark of woody plants usually cannot be compared with voucher specimens, but they can be taken into the field and directly compared with plants to be collected.

When a "re-collection" fails to reconfirm the activity of the original sample, the plant's identity may be questioned. The reference samples permit ready resolution of such a doubt.

Identification of Voucher Specimens

Every plant screened must be correctly identified. Many species are certain to be sampled several times. Extracts of a sample from one area will probably have the same activity as those of the same species collected elsewhere, and correct identification assures that all re-collections of an active species are assigned to the same laboratory for isolation of active agents. Thus, duplication of effort is avoided. After chemical structures are determined, they can be published under the correct plant name.

Many tools are available to assure correct identification. Modern floras provide keys and descriptions to permit accurate identification, and generic taxonomic monographs define morphologic limits of species and provide keys and descriptions to aid identification. However, knowledge of the world's higher plants is incomplete, particularly those of the tropical and subtropical areas that are rich sources of plant material. After many decades of botanical exploration, millions of specimens are in the major herbaria. These institutions usually specialize in

FIGURE 1.—Typical voucher specimen with field notes documenting two samples (left and right) collected for screening. Such vouchers are collected in sets of 5 so that duplicates will be available for deposit in several herbaria.

floras of specific regions and represent a vast reservoir of botanical information useful for resolving difficult identification problems.

Botanical Nomenclature

Once a plant is found to be active, taxonomic literature must be reviewed to assure that correct botanical nomenclature is applied. It is the task of the taxonomic botanist to verify the nomenclature of plants assayed in a screening program, correct botanical nomenclature being critical in a procurement program of such international scope. For example, camptothecin was first isolated from *Camptotheca acuminata* Decne. (family Nyssaceae) (2) and, more recently, from a plant identified as *Mappia foetida* (Wight) Miers (family Icacinaceae) (3). The identification of the plant was based on Hooker's *Flora of British India* published in 1875 (4), which describes four species, all from southern India. *Mappia foetida* was considered endemic to the Nilgiri Hills.

In 1845, Robert Wight, a British surgeon serving in India, first described the plant as *Stemonurus foetidus* Wight (5). Since 1845, specimens of this plant from Ceylon, southern India, Thailand, China, and Cambodia have been described as new species. Taxonomic literature includes 14 synonymous Latin binomials under four different generic names. Modern taxonomic research on the Icacinaceae has revealed that we are not dealing with a narrow endemic, but with a widely distributed species, *Nothapodytes foetida* (Wight) Sleumer. *Stemonurus* is now recognized as a separate Indomalaysian genus, while *Mappia* is recognized as a genus of New World plants (6).

Figure 2 shows the known distribution of *Nothapodytes foetida* and locations of suppliers in India, Thailand, and Taiwan who provide plant material for general screening. If a new sample from India is screened, it will probably be identified as *Mappia foetida*. This duplication will be recognized. However, if samples are later supplied from Thailand or Taiwan, they would undoubtedly be identified as

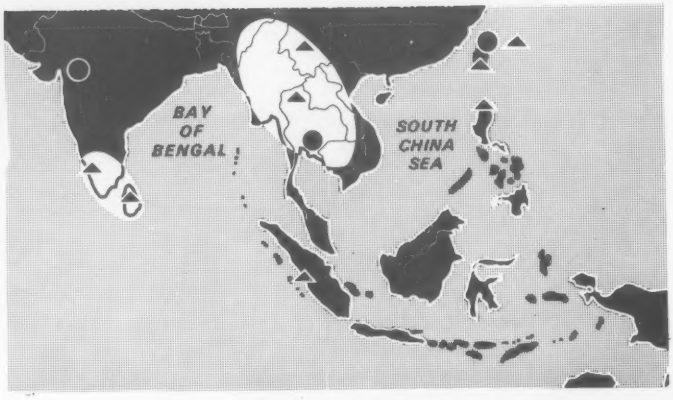

FIGURE 2.—Location of suppliers (dots) in India, Thailand, and Taiwan, and distribution of *Nothapodytes foetida:* the white areas are based on generalized field records and the triangles on precise locality records. (*Adapted from* ref 6.)

Nothapodytes foetida, since this species has been so distinguished in the *Flora of Thailand* (7) and in a modern treatise dealing with plants of Taiwan (8). Thus, without a nomenclature review, a re-collection might be assigned to a second laboratory for fractionation, duplicating research already completed.

Our present objective is to review the nomenclature of each new confirmed-active plant as soon as possible, so that when a re-collection is available for fractionation, we know the currently accepted name as well as any other names by which the plant has been known and under which it could also be undergoing fractionation.

Description of Samples

Plant samples are described to record as much information as possible about their content and the plants from which they were prepared. The abbreviations in table 1, used singly or in combination, adequately describe samples from almost any plant likely to be encountered. The rare exceptions can usually be described as "thallus" or body.

The system is flexible and, if followed carefully, usually indicates whether the plant is herbaceous or woody and, if herbaceous, much about its growth stage when the sample was prepared.

Sampling Plants for Screening

Success in the preliminary screening of plants for antitumor activity must be measured by the number and diversity of species for which antitumor activity can be demonstrated in relation to the total number of samples screened. The key to the discovery of a broad spectrum of useful antitumor agents from plants is to detect activity in as many species, genera, and families as possible. Once a species is known to produce a biologically active constituent it is a simple matter to determine which part of the plant is the best source.

TABLE 1.—Abbreviations used for describing plant samples

BU = bulb	RT = root
CO = corm	SB = stembark
EX = exudate	SD = seed
FL = flower	ST = stem
FR = fruit	TU = tuber
IF = inflorescence	TW = twig
LF = leaf	WR = rootwood
RB = rootbark	WR-RB = rootwood/bark
RH = rhizome	WS = stemwood
	WS-SB = stemwood/bark

When this program was initiated 15 years ago, the guideline stated that any plant part or combination of plant parts would be acceptable as a sample if the amount required for preparation of a suitable extract could be supplied. Many herbaceous plants will yield two different samples (eg, roots and leaves), most shrubs will yield three or more, and trees will yield six or seven. Following this guideline, a field project yielded an average of about four different samples for each species collected. From one viewpoint this is an ideal guideline, because the larger the average number of samples per species, the smaller the number of species that need to be sampled to reach a set quota and that must be documented in the field with notes and herbarium specimens and later identified. Also, with a large yield of samples from one field site, the botanist can devote more time to collecting and less time to searching for new collecting areas.

Some plant species concentrate useful chemical constituents in specific organs, eg, roots, stems, leaves, fruit, or seeds, while in others they are distributed throughout the plant. The antitumor agent maytansine is concentrated in the fruit of *Maytenus arbutifolia* (Hochst. ex A. Rich.) R. Wilczek, while nitidine, isolated from *Fagara macrophylla* (Oliv.) Engl., is concentrated in the rootbark. The antitumor activity of an African species of *Gnidia* is most highly concentrated in the leaves. However, camptothecin, from *Camptotheca acuminata*, is fairly well distributed through all parts of the tree except the leaves.

Herbaceous plants.—A comparison was made of two different methods of sampling herbaceous plants (9). The yield of active species from screening one group of samples, each representing the whole plant of a single species, was compared with the yield from a second group of samples in which each species was represented by two or three samples (eg, the roots separate from the above-ground part or parts). There seemed to be little difference in the yield of active species. It was concluded that, for herbaceous plants, the two procedures differed so little in their return on the screening investment, that if there was an important difference in yield it favored plants which had been separated into two or more samples.

Woody plants.—When samples were procured from areas where labor costs are low it became economically feasible to divide each species encountered into as many samples as possible. The only guideline available was the one originally established and the statistics based on herbaceous plants. Tree species, for example, were sampled to provide (*see* table 1 for abbreviations) WR, RB, WS, SB, TW, LF, and FR when available. Shrubby species usually provided WR-RB, WS-SB, TW, and LF. It soon became evident that the yield of activity from WS was quite low, and collection of these samples was discontinued except in two families, the Celastraceae and Simaroubaceae, whose active agents are commonly found in the WS. Enough data have now been accumulated to design a more rational sampling of woody plants. From a large collection of woody plant samples from Kenya, 94 yielded extracts which were active against PS leukemia and/or the KB cell line distributed as follows: LF (11); TW (18); SB (17); WS (12); WS-SB (eight); RT, WR, and RB (24); and other types (four). In this collection, which contained about an equal number of samples of LF and TW, the large number of active TW samples was impressive. There were fewer SB samples than LF or TW since many shrubby species represented by LF and TW were too small to supply SB. Therefore, the large number of active SB samples was also impressive.

These 94 active samples came from a total of 241 screened representing 44 active species, for most of which at least four samples were prepared. Experience with these woody plants suggests that different sampling guidelines might have yielded most of the active species that were identified under then-existing guidelines.

Table 2 shows the number of active species that would have been identified and those that would not have been identified under varying guidelines. Had sampling been limited to two per species, TW plus SB or WS-SB (SB preferred), 12 active species would have been missed. But 32 active species would have been identified at the cost of screening only 104 samples. It is evident that the loss would have been almost nil. Each of the genera listed in the third column of table 2 as "losses" include one or more confirmed-active species collected in Kenya or elsewhere in Africa that are likely to yield the same or closely related active compounds.

TABLE 2.—No. of active species that would have been and those that would not have been identified had other guidelines been followed in sampling woody plants from Kenya

Guideline	No. of active species	"Loss"
Standard	44	None
All parts acceptable except IF and FR	44	None
All parts acceptable except IF, FR, and LF	43	*Phyllanthus* sp.
All parts acceptable except IF, FR, LF, and WS	40	Above + *Acokanthera* sp., *Maytenus* sp., and *Terminalia* sp.
All parts acceptable except IF, FR, LF, WS, and RT (including WR and RB)	37	Above + *Croton* sp., *Euphorbia* sp., *Fagara* sp., *Gnidia* sp., *Melia* sp., *Rauwolfia* sp., *Tabernaemontana* sp., and *Uvaria* sp.
Limit sampling to 2 per species, either SB and TW, or WS-SB and TW	32	Same as above

The guideline suggested by the exercise with samples from Kenya was tested by a comprehensive analysis of a large lot of samples from Peru. This collection was prepared after a decision to avoid further testing of WS samples. These 3742 samples of woody plants fit into the categories indicated in table 3.

Table 3 shows, for samples of RT, WS-SB, SB, TW, and LF, the number of extracts assayed, the num-

TABLE 3.—Distribution of PS leukemia and/or KB cell line activity in aqueous-ethanol extracts of samples of Peruvian trees and shrubs

	Type of sample					
	RT	WS-SB	SB	TW	LF	Total
No. of extracts assayed	895	595	379	832	1041	3742
No. of active extracts	24	13	17	20	15	89
% active extracts	2.7	2.2	4.5	2.4	1.4	2.4

ber of active extracts, and the percent active against either PS leukemia, KB cells, or both. The data here generally agree with our experience in evaluating the woody plants of Kenya, ie, LF is a poor source of activity while SB is an excellent source. While TW does not appear to be as good, it seems superior to LF; RT is not as impressive as in the earlier exercise, nevertheless it is above average and second only to SB.

The 89 active extracts represent 67 species of woody plants. Had the revised guideline suggested by sampling woody plants of Kenya been followed, 45 active species would have been identified and 22 active species representing 16 genera would not have been identified. The screening program has identified active species in 11 of these 16 genera. Had the revised guideline been followed, only five active genera would have been lost.

Currently, samples of woody plants are limited to two for each species. Trees will be represented by TW and SB, while shrubs and trees too small to yield an adequate sample of SB will be represented by TW and WS-SB. One serious question is whether this new guideline should include testing of WS-SB. Future experience may show that it will be wise to avoid such samples, since the same activity may be shown by SB of related arboreus species. The new guideline will provide a much greater diversity of plants and should significantly increase the number of leads from the random-screening program.

RT and RB of woody plants will be eliminated for three reasons. First, when active agents are concentrated in the RT, they are likely to be present in sufficient amounts in TW, SB, or WS-SB to permit detection during screening. Second, RT and especially RB are the most costly part of the plant to collect. Finally, as a practical matter, a program must be conservation oriented. If a compound of interest is found only in the RB of a tree, problems in supplying sufficient amounts of raw material could be created, possibly destroying an unacceptable number of plants of a single species.

Selection of a Field Area

Kenya, in East Africa, has been a major source of plant material for the cancer chemotherapy screening program and is an excellent example of a nearly ideal field area, fulfilling many of the prerequisites for a successful field program. One of the most important factors to be considered in selecting a field area is availability of labor, since economic field programs can be achieved only with maximum use of carefully managed unskilled labor. Another factor is related to the ease with which the flora of the area can be identified. Procurement for a screening program should be carried out in areas where plants can be accurately identified with minimum delay. Knowledge of the Kenya flora is more than adequate and most of our collections can be identified at the East African Herbarium in Nairobi. Many problem specimens can be identified by the taxonomists now working on the new *Flora of Tropical East Africa.*

Useful tools for evaluating the potential of a country as a field area are maps and climatologic data. Figure 3 is a composite map adapted from the *National Atlas of Kenya* (10), which illustrates some of the matters discussed in the following paragraphs.

Among the important maps is one showing forest reserves. In these areas it is likely that a large diversity of plant species—trees, shrubs, and herbs—will be found within a relatively stable environment. In addition, forest officers, rangers, and guards who are acquainted with the forest are available to suggest areas where unusual plants are likely to be found.

Natural vegetation maps are of little practical value in planning a field program since they do not reflect alterations in the environment caused by man and his livestock. Even maps showing existing vegetation can be of limited use in a rapidly changing environment and are useful primarily from a negative point of view since they indicate which areas to avoid.

But even the richest forest is not suitable for an economic procurement program unless it is accessible. A map that shows the distribution of saw mills indicates utilization and roads that provide accessibility.

The physiographic map shows tropical coastal

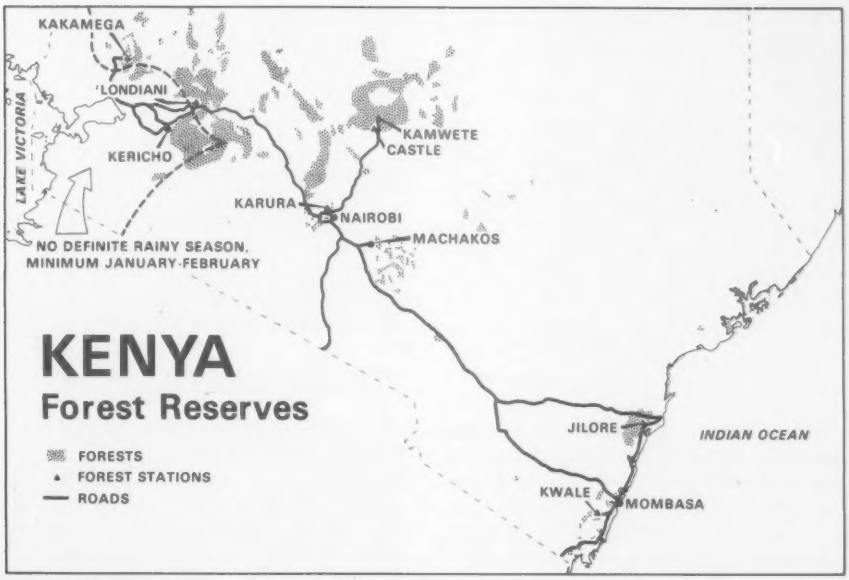

FIGURE 3.—Map of southern Kenya showing field locations selected to yield a maximum number and diversity of plant materials for screening. In western Kenya (the area to the left of the broken line) there is no definite rainy season. Showers are frequent and humidity is high throughout the year. Throughout the area to the right of the broken line there is at least 1 rainy season and 1 dry season. (*Adapted from* ref 10.)

lowlands, an interior highland with escarpments, mountain ranges, and high mountain peaks. Mt. Kenya, rising to an elevation of > 14,000 feet, is a few minutes south of the equator, which assures a wide variety of ecologic conditions which should yield great botanical diversity in the reserved forests. One face of the mountain is open to the sea, suggesting a wet side and a dry side, a further indication of diversity.

The environmental factor that predominates in the selection of a field area is rainfall. Low rainfall produces desert, scrub, grassland, and a paucity of species, while high rainfall assures adequate forest vegetation but also suggests difficulty in drying plant collections.

Most of Kenya has at least one marked dry season. In the eastern half of the country, there are two rainy seasons, the "large" and "small" rains. The monthly distribution of rainfall in Nairobi (fig 4), with highs in April and November and lows in January-February and July-August, is fairly typical of this region of the country. Thus, there are periods of heavy rainfall which produce lush vegetation, alternating with dry periods ideal for mobility over unpaved roads and tracts and for collecting and drying plant material.

Other factors must also be considered. There are two dry seasons and one is better than the other. During the "dry" months of July-August, the highlands often have a heavy cloud cover. Nairobi, for example, receives a mean of only a little more than 4 hours of bright sunshine each day (fig 5). With so little sunshine, the temperature is comparably lower and drying large masses of plant material can be very slow. However, at the height of the small

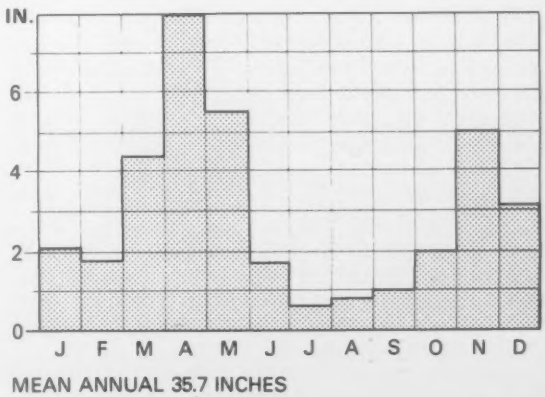

FIGURE 4.—Mean monthly rainfall distribution at Nairobi, Kenya. (*Adapted from* ref 10.)

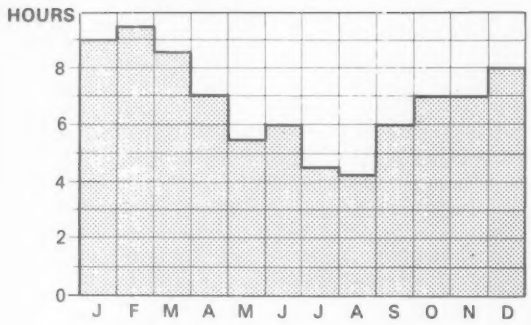

FIGURE 5.—Mean hours per day of bright sunshine at Nairobi, Kenya. (*Adapted from* ref 10.)

rains in November, Nairobi receives about 7 hours of sunshine, increasing to almost 10 hours/day by February. Thus, although January and February are the driest months of the year in Kenya, the collecting season begins in November since the long hours of bright sunshine make the moderate rainfall tolerable.

In western Kenya, the map shows areas of high rainfall but no definite dry season. At Kericho, with an annual rainfall of > 70 inches, daily showers are common even during the drier months. The good moisture conditions through the year result in a diversity of plants not found in the east, but also make drying the collections difficult. However, the community of Londiani, in a rain shadow, is only a 30-minute drive from Kericho and it is usually dry when Kericho is wet. There is a forest station at Londiani, making this an ideal base for field work in western Kenya.

Vegetation is more a product of climate than of geology and soils, but these factors must also be considered in the search for diversity. For example, the geologic map of Kenya shows isolated areas of granite south and southeast of Machakos isolated in an area of sedimentary basement system rocks. These are hill areas sufficiently elevated to draw enough precipitation to produce forest. Their isolation and distinctive rock formation suggest that there will be a number of species not likely to be encountered in other areas.

The soil map shows many different soil types in the Arabuko-Sokoke Forest, near the coast just inland from Malindi, which implies diversity of species. Such a lowland forest area is certain to yield many species not found elsewhere.

Two other factors, historical geology and climatology, are important in phytogeography and worthy of consideration in achieving the search for botanical diversification. But these need not receive serious consideration in the planning stage. In practice, the field botanist can learn best about what is most important—diversity—by discussing the phytogeography of an area like Kenya and its environs with botanists, foresters, and others who have had previous experience there.

Implementation of a Field Program

The plant procurement program requires field projects to supply three types of samples: (*a*) small samples (3 pounds each) for general screening; (*b*) large samples ("re-collections"), 50–100 pounds or more each, of confirmed-active plant material for fractionation; and (*c*) massive amounts of plant material, usually 10,000 pounds or more, needed as sources for isolation of larger quantities of compound(s) for preclinical and clinical evaluation. Each presents different problems and requires a different approach.

There are three primary keys to the success of any field project: (*a*) dependable transport, (*b*) maximum use and careful management of local labor, and (*c*) adequate supplies and equipment, especially for drying a large volume of plant material.

There are as many different approaches to the implementation of a field program as there are field botanists and field areas. The most successful program is one in which the field botanist applies a maximum of imagination and ingenuity in assembling, processing, drying, and properly documenting samples with minimal error in record keeping, often under adverse conditions. The most difficult field program to carry out is one designed to supply small samples for preliminary screening, since the number of plants sampled is great and each offers an opportunity for error in its documentation. Figure 6 illustrates one concept of an effective field team organized to assemble a maximum number of small samples for general screening in a minimum period of time. The botanical assistant and headman are experienced helpers who accompany the field botanist at all times. The botanical assistant's primary responsibility is to select plants for sampling, keep a mental record of species sampled to reduce duplication, and prepare herbarium specimens documenting species sampled. The headman closely supervises the collecting of samples to assure that the procedures followed will avoid errors, and also supervises processing, drying, and packing. The 16 figures in the lower half of the illustration represent local laborers who work under the supervision of the headman.

Samples of leaves, twigs, or fruit can be accurately labeled at the end of the day when they are assembled at the drying area by comparing them, if

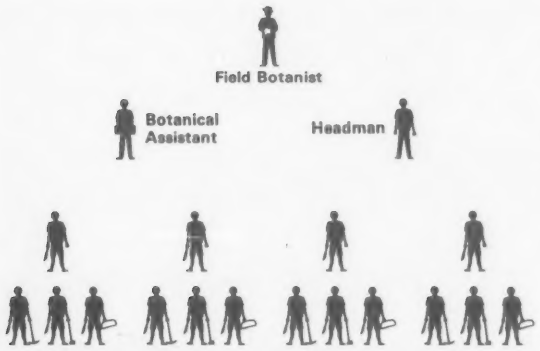

FIGURE 6.—An effective field team organized to assemble a maximum number of samples for screening in a minimum period of time.

necessary, with the voucher specimens documenting the plants sampled. Since samples of bark or roots cannot be matched with the voucher, a branch of the plant from which they were collected must be placed with such samples for identification until labels are attached.

Most samples are chopped with a compost mill adapted for this use and spread out to dry the same day they are collected. Chopping not only hastens drying, but greatly reduces bulk, which is essential since shipping costs are based on volume rather than weight.

In the field, productivity is most limited by the rate at which samples can be dried. Even though field work is scheduled for the dry season, the field botanist must be prepared to cope with unseasonable rains or, in some areas, very heavy dew (fig 7).

When the field project reaches its peak, as many as 250 samples can be drying at one time. They are left to dry as long as possible, but periodically some samples must be packed to make space for new ones.

The greatest efficiency is attained when a maximum number of samples are obtained from the environs (within about 25 miles) of a single field headquarters. Field activities must be highly concentrated geographically to assure a maximum return of plant material for general screening from each area sampled, in turn to assure a high concentration of active species which must later be collected in large quantity. In addition, time is valuable and much can be lost in moving from one location to another.

When a project is designed to secure large amounts of plant material of a single species, locating a plant in adequate numbers can present a real challenge. Every effort is made to obtain the recollection from the same site as the original active collection and during the same season.

Reconnaissance is a necessary preliminary to a re-collection project, especially when a massive quantity of plant material is required. In 1972, a massive quantity of woody stems of *Maytenus buchananii* (Loes.) R. Wilczek was required as a source of maytansine for preclinical and clinical evaluation. The plant is a scandent shrub with twisted stems up to about 4 inches in diameter which relies on other vegetation for support until a few branches reach the forest margin or canopy. Although widely distributed in Africa, it is not common at any one place. Herbarium records suggested the plant might be most readily available in the Shimba Hills, just inland from the Kenya coast. After a 2-day reconnaissance it was evident that the plant grew along forest margins where it received some light and also the support of other vegetation. Thus, it would probably be most abundant in isolated nonreserved forest areas where most of the larger trees had been removed for timber. The most accessible forest fitting these specifications was inspected, and the plant was found in great abundance.

Once found, four problems were obvious: managing the large labor crew that would be required to assure collection in a short period, preventing adulteration, chopping to reduce bulk, and drying.

Proper supervision to assure prompt collection of the required quantity of the correct plant was made difficult by the number of laborers involved. To assure productivity, bonuses were paid to workers who assembled the largest amount of material.

Since the stems of this plant have two distinct characteristics that easily distinguish it from all other similar plants in the forest (a thin yellow layer just under the grey outer bark and a bright reddish inner bark), it was possible to reveal adulterants in the collected material by slashing the stems with a large knife.

A wood chipper was used for reducing the bulk of the collected material, which was then hauled to a desert area for drying.

Contract Procurement

Since the screening program expanded in July 1971, procurement has been increasingly accomplished by contracts with suppliers in foreign countries. Figure 8 shows the general locations of dependable sources of supply. The arrows, from Hong Kong to Djakarta, show access to medicinal plants of China through commercial sources. Some of the dots on the map represent more than one source. The supplier network is being extended to cover new phytogeographic areas to increase the probability of having a source of supply for any plant needed for the cancer chemotherapy program and for other

FIGURE 7.—Drying area, Jilore, Kenya. This site is just inland from the coast and even during the dry season is subject to frequent light and occasional heavy showers. The rope networks support large tarpaulins used to cover the drying "beds" at night and during showers. Ditches between and around the drying beds assure runoff during heavy showers. The windbreak in the background was constructed from poles assembled from the forest nearby and large palm-like leaves of the cycad, *Encephalartos hildebrandtii*. A large tarpaulin used to protect samples from rain is being removed. The facility is being used to dry small samples for general screening.

natural-products screening programs that will follow. The map shows areas where new sources will be sought.

Currently, all plant material for preliminary screening is obtained from contract sources. The map shows four countries (Colombia, Mexico, Kenya, and Tanzania) where field programs are conducted by Laboratory botanists.

The Hazard of Insect Pests

Any large-scale plant procurement program of such international scope creates a potential hazard from pests, especially insects. This hazard is probably minimal because most imported materials are from tropical areas and insects that may infest these plants could probably not survive in the US. Nevertheless, great caution must be exercised. Work areas where shipments of plant material are received and unpacked are closely monitored by APHIS to determine if potentially hazardous insects are present. When infested samples are noted, they are enclosed in plastic bags and insect specimens are sent to specialists for identification. Usually, such insects are already present within the US. Rarely, insects are identified that might be a threat, and the infested samples are fumigated or burned.

PRESENT AND PROSPECTIVE SOURCES OF SUPPLY

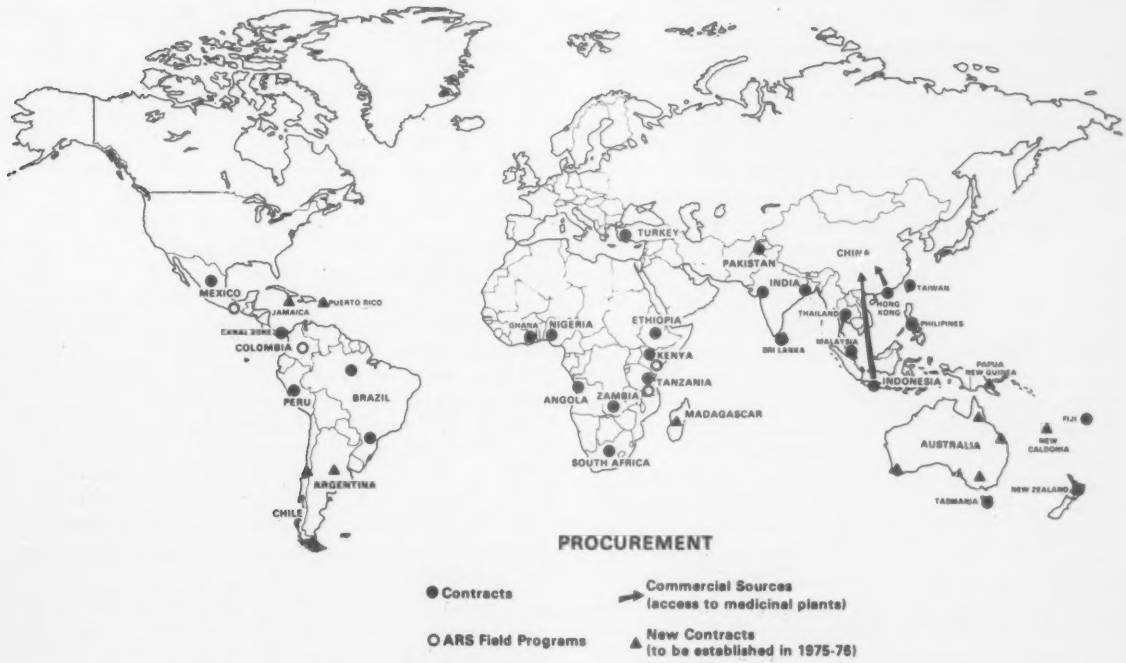

FIGURE 8.—Plant material for the screening program is now obtained largely from suppliers in foreign countries. The arrows reflect access to medicinal plants of China through commercial sources in Hong Kong and Indonesia.

Fumigation is objectionable because the most commonly used fumigants are reactive and may alter chemical compounds in plants; they are also pollutants. A new receiving facility will be equipped with a large walk-in freezer where infested samples can be stored at very low temperature to destroy any imported insects which could become pests in this country.

Random Versus Rational Selection of Plants for Screening

After 15 years of screening, we can reliably predict that some plant families will be good sources of antitumor activity and others will be poor sources. When procurement is conducted purely at random, about 25% of the plants screened will be members of the families Poaceae, Asteraceae, and Fabaceae. The family Poaceae has been a poor source of activity, and though there have been many active species of Asteraceae and Fabaceae, active agents isolated from plants in these families have not been promising. Activity of many species of Asteraceae, for example, has been due almost exclusively to sesquiterpene lactones, compounds usually active only against KB cells; the few species active against solid tumors have therapeutic indexes too low to permit use as antitumor agents. Because they have shown so little potential, procurement of other species in these families has been discontinued.

Screening has also identified several plant families as very good sources of activity: Apocynaceae, Celastraceae, Rutaceae, Simaroubaceae, and Thymelaeaceae. For the past several years, many representatives of these families have been procured in large lots of 50–100 pounds so that fractionation to isolate active agents could proceed immediately after activity was demonstrated.

This type of procurement has both advantages and disadvantages. In many cases, active agents isolated from these plants are likely to be identical

to compounds previously identified as antitumor agents. For example, nitidine is the active agent responsible for antitumor activity of two species of Rutaceae, *Fagara macrophylla* and *Toddalia asiatica* (L.) Lam. In other cases, new active compounds are likely to be similar but superior to those previously isolated. Of two species of Simaroubaceae, bruceantin from *Brucea antidysenterica* J. F. Mill. is superior to holacanthone from *Holacantha emoryi* Gray. However, some plant families are so diverse that new active compounds are likely to be totally different from those previously isolated. The family Celastraceae is a good example because it is the source of maytansine isolated from species of *Maytenus* and *Putterlickia* and triptolide isolated from *Tripterygium wilfordii* Hook. fil. Maytansine has been approved for clinical evaluation and triptolide is a very strong candidate.

The greatest achievement of this guided procurement program has been the identification of better sources of maytansine, a compound isolated originally from fruit of *Maytenus arbutifolia* from Ethiopia. By the time a supply of the compound was needed for preclinical and clinical evaluation, it was known that the wood of *Maytenus buchananii* from Kenya was a better source. More recently, *Maytenus buchananii* from southern Tanzania has proven superior to the Kenya plant, and this plant has been superseded in yield of maytansine by *Putterlickia verrucosa* (E. Mey. ex Sonder) Szyszyl. from South Africa.

The Role of Conservation in a Procurement Program

Since it is imperative to assure future supplies of plant materials that will be needed in large quantity, it is necessary to be very conservation conscious.

In 1966, there was concern in Wisconsin when a large supply of roots of meadow rue (*Thalictrum dasycarpum* Fisch., C. A. Mey. and Ave-Lall.) was needed to supply thalicarpine for further evaluation. Anticipating that an even larger amount of thalicarpine might be needed in the future, and following the general rule that chemical compounds are often more highly concentrated in seed than in other parts of the plant, field surveys were conducted in 1967, 1969, and 1971 to collect seed and also to locate good stands of meadow rue. Analyses conducted by S. Morris Kupchan confirmed that seeds were superior to roots as a source of thalicarpine, and a massive field program in 1973 yielded > 6000 pounds of seed without adversely affecting the natural population.

When in 1972, 20,000 pounds of the woody stems of *Maytenus buchananii* was needed as a source of maytansine, there was alarm on the part of conservationists in Kenya. There was reason for this concern, since herbarium records suggested that this species was uncommon if not rare. In early 1974, 2 years after the massive collection, there was little evidence of regeneration of the plant in the forest. If maytansine becomes a useful drug, the forests of Africa will not yield an unlimited supply. Fortunately, this species is easy to propagate from either cuttings or seed. Plants flower early and mature seeds that germinate easily are produced within about a year. This plant produces a heavy crop of fruit and we believe that the seed will be a superior source of maytansine. *Maytenus buchananii* can be established as a crop and agriculture can produce the necessary raw material.

We are much concerned about conservation of our natural resources, and our concern extends beyond esthetics and environmental protection. Without conservation of these resources, future natural-products screening programs might be limited to microorganisms, cultivated plants, weeds, and animal organisms.

REFERENCES

1. STATZ D, and COON FB. Preparation of plant extracts for anticancer screening. Cancer Treat Rep 60:999–1005, 1976.
2. WALL ME, WANI MC, COOK CE, ET AL. Plant antitumor agents. I: The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890, 1966.
3. GOVINDACHARI TR, and VISWANATHAN N. 9-Methoxycamptothecin. A new alkaloid from *Mappia foetida* Miers. Indian J Chem 10:453–454, 1972.
4. MASTERS MT. *In* Flora of British India (Hooker JD, ed). London, L. Reeve and Co, 1875, vol 1, pp 588–589.
5. WIGHT R. Icones Plantarum Indiae Orientalis or Figures of Indian Plants. Madras, Athenium Press, 1845, vol 3, part 33, plate 955.
6. SLEUMER H. Materials towards the knowledge of the Icacinaceae of Asia, Malesia, and adjacent areas. Blumea 17:181–264, 1969.
7. SLEUMER H. Icacinaceae. Flora Thailand 2:75–92, 1970.
8. LI HL. Woody Flora of Taiwan. Narberth, Pa, Livingston Publishing Co, 1963.
9. PERDUE RE, JR, ABBOTT BJ, and HARTWELL JL. Screening plants for antitumor activity. II. A comparison of two methods of sampling herbaceous plants. Lloydia 33:1–6, 1970.
10. National Atlas of Kenya, 3rd ed. Survey of Kenya. Nairobi, 1970.
11. PERDUE RE, JR. Processing plant samples with a compost mill to hasten drying and reduce bulk. Econ Bot 23:277–287, 1969.

Preparation of Plant Extracts for Antitumor Screening[1]

DuWayne Statz* and Francis B. Coon[2]

SUMMARY

Random collections of plants have been screened by the National Cancer Institute's screening program for almost two decades. The WARF Institute, Inc (Madison, Wis) has been preparing most of the crude plant extracts for antitumor screening for the past 14 years. This paper describes our present method of extraction for preparation of crude plant extracts for screening and presents a history of past procedures used for the extraction of plant materials. Wherever possible, screening statistics have been included for plants which have been extracted by the various procedures.

[Cancer Treat Rep 60:999–1005, 1976]

Plant products were introduced during 1957 into the NCI screening program. Due to the increased interest in the screening of plant materials in the late 1950s, the WARF Institute, Inc, contracted with the NCI in August 1961 to prepare crude plant extracts. Since 1961, we have extracted approximately 35,000 plant samples for antitumor screening. The majority of these plant samples have been procured by the Medicinal Plant Resources Laboratory, US Department of Agriculture (Beltsville, Md) (1).

During the 18-year period in which plants have been studied in the cancer screening program, only a few major changes have occurred in extraction procedures. Before describing the present extraction procedure it is helpful and interesting to look at the evolution of the procedures that have been used over the last 18 years.

During the 4-year period between 1957 and 1961, plant extracts were being prepared at the University of Wisconsin, the University of Arizona, and Chas. Pfizer and Co (Maywood, NJ). The extraction procedure developed by Dr. Koppaka Rao (Chas. Pfizer and Co) after 16 years of experience working with plant materials is shown in figure 1. Most plant extracts prepared by this method were screened against the rat tumor Walker 256 carcinosarcoma. Although the total number of plant extracts prepared by this method is unknown, confirmed activity against the Walker 256 tumor was found in the water fraction of 44 plant samples, in the petroleum ether fraction of 13 plant samples, and in the insoluble fraction of 22 plant samples.

The procedure followed by Dr. Jack Cole (University of Arizona) is outlined in figure 2. According to this method, a 50% chloroform-ethanol and a water extract were prepared from each sample. Approximately 4500 plant extracts were prepared using this procedure, but the number and distribution of confirmed active extracts were not recorded.

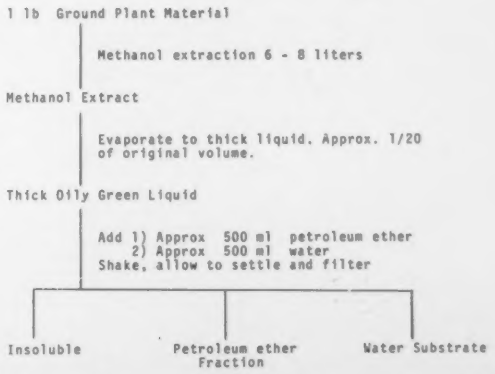
1 lb Ground Plant Material
Methanol extraction 6 - 8 liters
Methanol Extract
Evaporate to thick liquid. Approx. 1/20 of original volume.
Thick Oily Green Liquid
Add 1) Approx 500 ml petroleum ether
2) Approx 500 ml water
Shake, allow to settle and filter
Insoluble
Petroleum ether Fraction
Water Substrate

FIGURE 1.—Extraction procedure for plant materials developed by Dr. Koppaka Rao, Chas. Pfizer and Co.

[1]Supported by contract N01-CM-43707 from the Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

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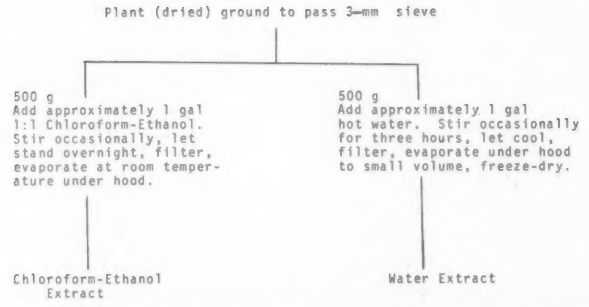
Plant (dried) ground to pass 3-mm sieve
500 g
Add approximately 1 gal 1:1 Chloroform-Ethanol. Stir occasionally, let stand overnight, filter, evaporate at room temperature under hood.
500 g
Add approximately 1 gal hot water. Stir occasionally for three hours, let cool, filter, evaporate under hood to small volume, freeze-dry.
Chloroform-Ethanol Extract
Water Extract

FIGURE 2.—Extraction procedure for plant materials developed by Dr. Jack Cole, University of Arizona.

Dr. Morris Kupchan (University of Wisconsin) began preparing plant extracts for antitumor screening in 1958 according to the procedure shown in figure 3. This procedure, which involved preparing a water and an alcohol extract, was adopted by us when we entered the program in 1961. By 1964, >9000 plant samples had been processed. Activity against the Walker 256 carcinosarcoma was confirmed in approximately 10% of these plant samples.

All of the extracts that we prepared using this procedure were also screened against the in vitro 9KB tumor. This tumor was originally taken from a human epidermoid carcinoma of the nasopharynx and is still being maintained today as the primary in vitro screen for all plant extracts. When this procedure was re-examined, it was proposed that the same spectrum of chemically active substances could be extracted from plant materials using a 50% mixture of water and ethyl alcohol. A single-solvent extraction would also have the benefit of doubling the available screening capacity. In 1963, our institute carried out a trial run using the aqueous-ethanol mixture. The experiment confirmed the hypotheses and the single-solvent extraction procedure (fig 4) was adopted in March 1964. By October of the same year, 1254 plants had been prepared by the single-solvent procedure and screened against the Walker 256 tumor. Antitumor activity was confirmed in 9% of the plants screened.

Since it had several advantages, the aqueous-ethanol procedure became the procedure of choice for the next 10 years. It had the capability of extracting a broad spectrum of chemical substances and required that only a single extract be screened. The majority of extracts were dry powders easily prepared in the screening laboratories for injection into test animals. At the same time, the simplicity of this procedure allowed our personnel to process a large number of plant samples in a short period of time. During 1972 and 1973, plant samples were being processed by this method at a rate of approximately 175 samples per week.

During the 10-year period in which this procedure was used, several changes were made in the in vivo screening protocols which necessitated a re-evaluation of the extraction procedure. The most important changes were those in the tumor systems used for the primary screen. It became apparent that the Walker 256 tumor was not selective enough in its response. Many of the plants active against the

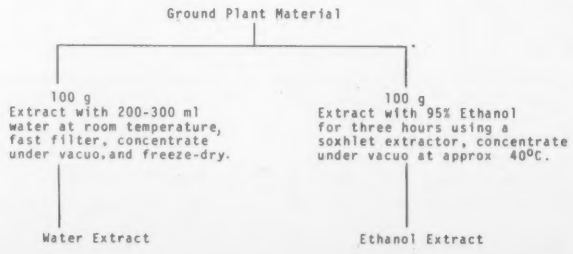
Ground Plant Material
100 g
Extract with 200-300 ml water at room temperature, fast filter, concentrate under vacuo, and freeze-dry.
100 g
Extract with 95% Ethanol for three hours using a soxhlet extractor, concentrate under vacuo at approx 40°C.
Water Extract
Ethanol Extract

FIGURE 3.—Extraction procedure for plant materials developed by Dr. Morris Kupchan, University of Wisconsin.

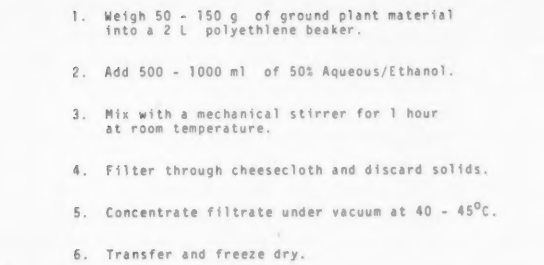
1. Weigh 50 - 150 g of ground plant material into a 2 L polyethlene beaker.
2. Add 500 - 1000 ml of 50% Aqueous/Ethanol.
3. Mix with a mechanical stirrer for 1 hour at room temperature.
4. Filter through cheesecloth and discard solids.
5. Concentrate filtrate under vacuum at 40 - 45°C.
6. Transfer and freeze dry.

FIGURE 4.—Extraction of plant materials with 50% aqueous-ethanol solution.

Walker 256 tumor were found to be inactive or, at most, marginally active after fractionation or isolation studies. Therefore, the preliminary in vivo screen was changed in 1969 so that all plant extracts were tested against two murine leukemias, L1210 and P388. In 1972, data were compiled on 12,000 plant samples which had been screened against the two leukemias after extraction with the 50% aqueous-ethanol mixture (table 1). The number of crude extracts which demonstrated activity against the L1210 tumor was insignificant, with only one sample out of 12,000 with confirmed activity. On the other hand, activity against the P388 tumor was confirmed in 361 (or 3%) of the crude extracts tested. In subsequent fractionations of extracts active against P388, 28 of 361 demonstrated L1210 activity. As a result, the routine screening against L1210 was eliminated.

Since 1972, all crude plant extracts have initially been screened against the P388 tumor. Samples with confirmed activity against P388 are subsequently screened against L1210 both as crude extracts and as fractions obtained from the isolation studies. All of the plant extracts we prepared have also been screened against the in vitro 9KB tumor. Of the 41,074 extracts so screened, 632 (or 1.53%) confirmed activity in this tumor system.

About the same time that the L1210 and P388 data were compiled in 1972, it was proposed that the percentage of plants with confirmed activity against these tumors could be significantly increased if the plants were subjected to a simple solvent fractionation before screening.

Therefore, in 1972 an experimental procedure was developed under the direction and guidance of Dr. Jonathan Hartwell (then Head, Natural Products Section, NCI). The schematic diagram for this procedure is shown in figure 5. It was decided that the WARF Institute would pilot 500 samples through the fractionation procedure in a study termed the "500 Plant Experiment." For comparative purposes each of the plants was also extracted by the aqueous-ethanol procedure. A total of 504 plants, randomly selected by the Medicinal Plant Resources Laboratory, were actually processed in this study.

The extraction of the "500 Plant Experiment" samples was completed during the spring of 1974. By May of 1975, approximately 90% of the extracts had completed the P388 protocol and nearly all had completed the 9KB protocol. An analysis of the "500 Plant Experiment" data accumulated until May 1, 1975, is shown in figures 6 and 7.

Figure 6 represents a direct comparison of the fractionation procedure and the 50% aqueous-ethanol procedure. Of the plants processed by the fractionation procedure, 21.4% had confirmed activity against P388 in contrast to 0.8% of the plants processed by the aqueous-ethanol procedure. The

TABLE 1.—Yield of L1210-confirmed active materials (T/C% ≥ 125) from crude plant extracts screened against both L1210 and P388 (ref 2)

	No. of active extracts/total No. tested	%
Crude plant extracts		
Tested in L1210 and P388	Approximately 12,000	—
Confirmed in L1210 and P388	1/12,000	<0.01
Confirmed in L1210 only	0/12,000	0
Confirmed in P388 only	361/12,000	3.0
P388 actives (crude) confirmed in L1210 during fractionation	28/361	7.8
Yield of L1210 active materials resulting from P388-L1210 dual screen	28/12,000	0.23
L1210 actives, accumulated through Jan 1972, from screening of crude plant extracts against L1210 only	15/49,290	<0.03

data for the in vitro screening are similar although the difference is not quite as dramatic; 5.4% of the plants processed by fractionation were active compared to 2.4% processed by the aqueous-ethanol procedure.

Figure 7 shows the relative distribution of activity among the different solvent fractions. Most of the antitumor principles were concentrated in the 95% ethyl-alcohol extract and in the 90% methyl-alcohol fraction of that extract. Few active compounds were found in the water fraction, and almost none were concentrated in the hexane fraction.

The L1210 data have been omitted from both of these figures. At the time these data were last evaluated, approximately 50% of the extracts which had confirmed activity against either the P388 or 9KB tumors had completed the L1210 protocol. Although two of these extracts demonstrated activity against L1210, both must be re-tested in this system before they are considered L1210 active.

As a result of the preliminary data from the "500 Plant Experiment," the routine procedure of processing all new plants with the aqueous-ethanol mixture was discontinued in May of 1974, and since then all new plants have been subjected to the fractionation procedure.

As a consequence, the procedure used in the "500 Plant Experiment" was modified slightly to make it more suitable for extracting large numbers of plants. Figure 8 shows the schema for this modified procedure which is the method we presently use. It is essentially the same in principle as the procedure used in the "500 Plant Experiment," the main difference being that the plant material is initially defatted with petroleum ether. This has eliminated problems with emulsions forming in the chloroform-water partition and has also eliminated the 90% methyl alcohol-hexane partition. For safety purposes, the ground plant material is now being processed in soxhlet extractors rather than open beakers.

During 1974, our laboratory was adapted for application of the modified procedure on a routine scale. A series of known active plants were first piloted through the modified procedure in order to double check the legitimacy of the modifications. To date, most of the screening has been completed on the extracts from these active plants and it appears that the procedure is working as well as expected.

By late 1974 we were extracting approximately 45 new plant species per week. By May of 1975, 1700 different plant species had been extracted by this new method. Unless additional changes are made, it is expected that 7000–7500 new plant species will be extracted by this procedure at our institute over the next 3 years.

The modified fractionation procedure is the most recent modification of the procedures used to prepare plant extracts for the preliminary evaluation of their anticancer activity. These changes have occurred gradually, with the goal of developing a procedure that would give the highest percentage of confirmed actives for a given collection of plants when screened against a particular tumor. At the same time, procedures have been utilized that would allow the extraction of as many different plant samples as possible in the shortest period of time.

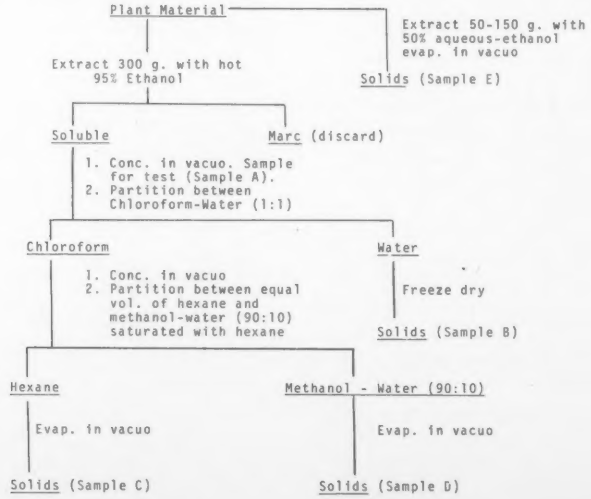
Plant Material
Extract 50-150 g. with 50% aqueous-ethanol evap. in vacuo
Extract 300 g. with hot 95% Ethanol
Solids (Sample E)
Soluble
Marc (discard)
1. Conc. in vacuo. Sample for test (Sample A).
2. Partition between Chloroform-Water (1:1)
Chloroform
Water
1. Conc. in vacuo
2. Partition between equal vol. of hexane and methanol-water (90:10) saturated with hexane
Freeze dry
Solids (Sample B)
Hexane
Methanol - Water (90:10)
Evap. in vacuo
Evap. in vacuo
Solids (Sample C)
Solids (Sample D)

FIGURE 5.—Procedure for "500 Plant Experiment."

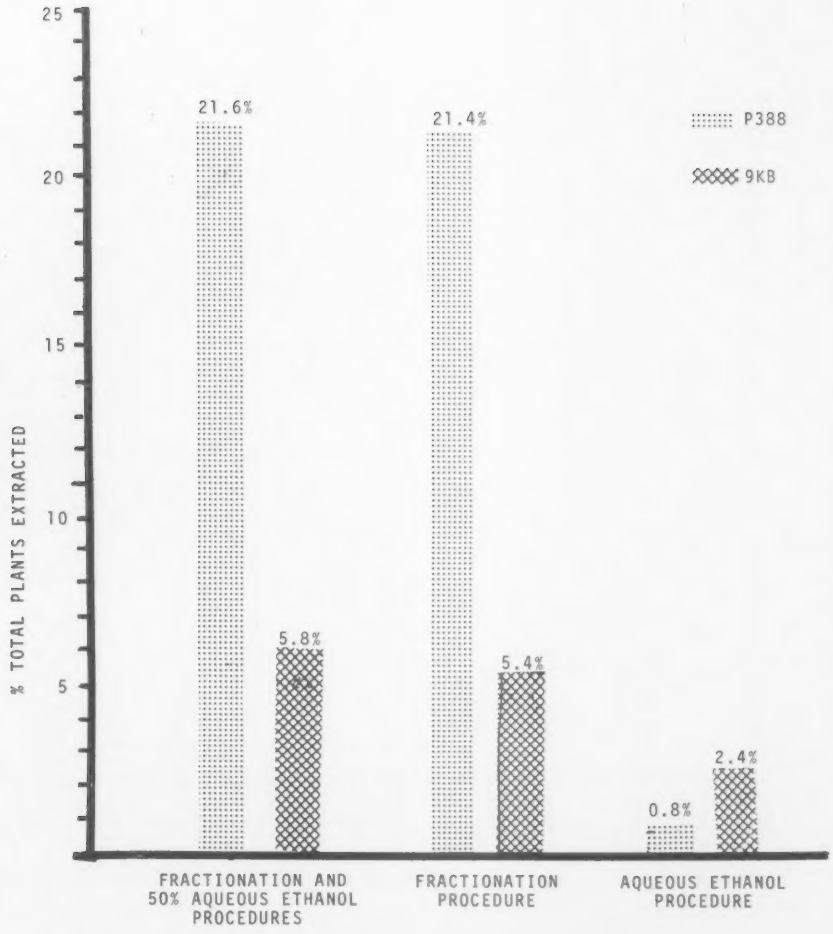

FIGURE 6.—"500 Plant Experiment"—comparison of 50% aqueous-ethanol and fractionation procedure data to May 1, 1975 (504 plants extracted).

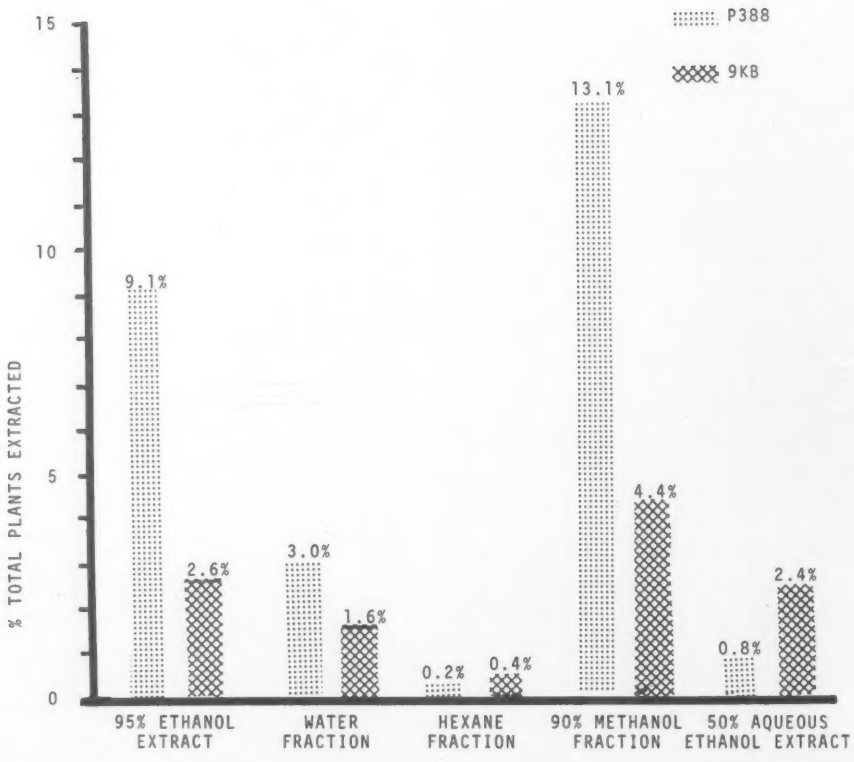

FIGURE 7.—"500 Plant Experiment"—breakdown of confirmed active extracts to May 1, 1975 (504 plants extracted).

Plant Material
1. Extract with Skellysolve B, 60-68°C (via Soxhlet, 8 hours)
2. Discard petroleum ether extract
3. Air dry plant material
Extract with 95% reagent ethanol (via Soxhlet-14 hours)
Soluble
Marc (discard)
1. Conc. in vacuo to ca. 100 ml. Take 1/4 and conc. to paste in vacuo (Sample A)
2. Conc. remainder to paste in vacuo and partition between Chloroform - Water (1:1)
Water (discard)
Chloroform
Evap. in vacuo
Solids (Sample B)

FIGURE 8.—Modified fractionation procedure for plant materials.

REFERENCES

1. PERDUE RE, JR. Procurement of plant materials for antitumor screening. Cancer Treat Rep 60:987–998, 1976.
2. VENDITTI JM. Foreword. *In* Geran RI, Greenberg NH, Macdonald MM, et al. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). Cancer Chemother Rep Part 3, vol 3(2):1–5, 1972.

Magnification of Plant Parts:

♀habit x ½
flower x 7½
fruit x 2 (profile view and opened to show the pendulus seed)
seed x 2

♂(upper left)
flower x 7½ (top, natural appearance; below, flower spread to show parts)
petals x 7½ (note hairs inside both petals and sepals)
stamens x 15 (note minute hairs)
inflorescence x ½

Brucea antidysenterica Mill.—The source of bruceantin, this is a small tree, rarely > 14 feet high with stems up to about 2 inches in diameter. It is widely distributed through the highlands of tropical Africa, but abundant apparently only in Ethiopia. The active agent is concentrated in the stembark.

Bioassay of Plant Extracts for Anticancer Activity

B. J. Abbott[1,*]

SUMMARY

Crude plant products were first tested in the Drug Research and Development Program, Division of Cancer Treatment, NCI, in 1956. The first materials were voluntarily submitted and later materials were acquired through various contracts. A review is given of the tumor systems which have been used for the evaluation of these plant products and of in vitro assays utilized to expedite fractionation and isolation.

[Cancer Treat Rep 60:1007–1010, 1976]

The first crude plant product was tested in the Drug Research and Development Program (DR&DP), Division of Cancer Treatment, NCI, in 1956. The testing was carried out in three transplantable tumor systems: two solid tumors, the Sarcoma 180 (S180) and the Adenocarcinoma 755 (CA-755), and in an ascitic tumor, L1210 leukemia. These tumors were selected by the screening panel of the Cancer Chemotherapy National Service Center, now known as the DR&DP, based upon the experience and knowledge available to this panel in 1955, with great importance placed upon the ability of these screens to select almost all of the materials then considered to be clinically useful in man.

For routine screening the solid tumors were implanted sc as fragments on what was defined for test purposes as Day 0. Drug treatment was begun 24 hours after tumor implantation and was continued for 7 days in S180 and for 11 days in CA-755. Twenty-four hours after the last treatment the tumors of the control and test animals were excised and weighed. The ratio of the mean tumor weight of treated animals to that of controls (T/C) and body weight changes of the mice in the treated and control groups were recorded. For testing plant extracts a two-stage screening sequence with S180 and CA-755 was used and employed the following acceptance limits:

Stage 1	$(T/C)_1 \geq 0.45$	Reject
	$(T/C)_1 < 0.45$	Continue
Stage 2	$(T/C)_1 \times (T/C)_2 \geq 0.2$	Reject
	$(T/C)_1 \times (T/C)_2 < 0.2$	Accept

L1210 leukemia cells were transplanted ip using ascitic fluid from donor mice diluted to a concentration of 10^5 cells/ml. The mean survival time (MST) of the animals was recorded and a T/C value was calculated as the ratio of the MST of the test animals to that of the control animals expressed as a percentage. At this period in the program a two-stage test was also used for L1210 leukemia which had the following limits:

Stage 1	$(T/C)_1 < 1.25$	Reject
	$(T/C)_1 \geq 1.25$	Continue
Stage 2	$(T/C)_1 \times (T/C)_2 < 1.56$	Reject
	$(T/C)_1 \times (T/C)_2 \geq 1.56$	Accept

For all three systems a confirmation procedure was used for plant extracts based on the two-stage sequence. This required that a fresh preparation of the extract be tested at four dose levels with doses used encompassing the active dose range seen in the two-stage sequence test (1). According to our records the first plant extract tested was a methanol extract of seeds from *Phytolacca americana* L. (NSC-3672). It was negative in all three systems tested (1). From this beginning the testing of crude natural products has increased dramatically to the extent that if punch cards of the natural products file were compared today with the 550-foot Washington Monument, the 1,004,218 records would tower to 837 feet (2).

The screening program progressed and it has

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now been diversified to take into account knowledge gained as to new tumor systems as well as to the clinical efficacy of compounds previously screened. The 9KB system was introduced as an in vitro screen for plant products in late 1960 and assaye according to the method developed by Eagle and Foley (3–5) and modified by Smith et al (6,7). It has proven useful in following the fractionation and isolation of plant products in those instances in which there is correlation with in vivo activity.

P388 and L1210 leukemias in culture have also proven worthwhile for this purpose where insufficient activity was demonstrated against the KB system (8,9).

Other in vitro systems evaluated for potential usefulness as indicators of antitumor activity have included various microorganisms such as the mold *Neurospora crassa* (10), *Escherichia coli*, *Lactobacillus casei*, and *Tetrahymera pyriformis* (11); such systems were judged to be less useful.

By 1962 protocols for screening included a battery of 23 tumor systems and plant extracts were routinely tested against two randomly selected tumor systems in addition to L1210 (12). The tumor systems most commonly used in addition to S180, CA-755, and L1210 and the parameter used for evaluating antitumor activity are shown in table 1. In 1966 this screening program was evaluated as to its ability to uncover clinically effective agents by Goldin et al (13). Based on this evaluation, a program decision was made to test all materials, when a sufficient quantity was available, in two tumor systems, the L1210 leukemia and the Walker 256 carcinosarcoma (W256). The latter tumor was transplanted by an im injection of 0.22 ml of a one to six dilution of tumor homogenate into the thigh of non-inbred Albino rats. Treatment was initiated 3 days after tumor implant and was administered daily for 4 days. Animals were killed on the seventh day after tumor implantation. The antitumor activity of the test compound was assessed by comparing the weights of tumors of test animals with those of control animals. Two purified plant products (thalicarpine [NSC-68075] and *d*-tetrandrine [NSC-77037]) selected on the basis of their activity against this tumor system are currently in clinical trial.

TABLE 1.—Commonly used tumor systems and parameter used for evaluation of activity

Test code	Tumor	Parameter
5WA16	W256 im	Mean tumor weight
5DL31	Dunning ascitic leukemia	Median survival time
8H1	HS1 human sarcoma in egg	Mean tumor weight
7D1	Adenocarcinoma, duodenum	Mean tumor weight
3FV	Friend solid virus leukemia	Mean tumor weight
3LL12	Lewis lung carcinoma	Mean tumor weight

TABLE 2.—Comparative sensitivity of ip L1210 and P388 leukemias to 10 plant products

Plant product	% increase in lifespan (ILS) over controls*	
	L1210	P388
Vinblastine	40	112
Vincristine	47	142
Emetine	40	100
Ellipticine	72	104
Camptothecin, sodium	100	95
Harringtonine	45	116
Indicine-*N*-oxide	40	100
VM-26	200	150
Podophyllotoxin	N†	52
Demecolcine	39	50

*Drug test values shown in this and subsequent tables represent pooled data reported to the Drug Evaluation Branch, DR&DP, DCT, NCI, from contract laboratories. The % ILS over controls is the mean of the maximum observed in each dose-response test in which an optimal dose was clearly attained. Each individual ILS value is the mean value from tests using the same treatment schedule, but the treatment schedule may not have been identical for all drugs. In general, for each drug, the treatment schedule for L1210 and P388 activity was the most effective of the schedules that would be feasible for large-scale screening. L1210 or P388 was implanted ip on Day 0. The treatment schedules used were 1 treatment on Day 1 only; 1 treatment on Days 1, 5, and 9; or daily treatment from Day 1 through Day 9 or 10 (*from* refs 15 and 16).

†N = %ILS < 25.

TABLE 3.—Comparative sensitivity of ip L1210 and P388 leukemias to 16 fermentation products

Fermentation product	% ILS over controls*	
	L1210	P388
Adriamycin	64	> 200
Daunorubicin	58	127
Bleomycin	N	50
Actinomycin D	45	> 175
Mitomycin C	70	150
Mithramycin	N	122
Streptonigrin	N	36
Azotomycin	68	120
Streptozotocin	60	54
L-Asparaginase	N	36
Azaserine	50	97
Chromomycin A_3	N	153
Neocarzinostatin	63	> 200
Tubercidin	N	50
Sangivamycin	67	90
Streptovitacin A	55	81

**See* footnotes in table 2.

TABLE 4.—Activities of natural products reported to have some clinical activity

NSC No.	Name	Mouse L1210 leukemia, ip* (% ILS)	Rat W256, im† (% tumor weight index [TWI])	Mouse P388 leukemia, ip‡ (% ILS)	Rat Dunning ascitic leukemia, ip§ (% ILS)	Mouse CA-755, sc¶ (% TWI)
49842	Vinblastine	40**	89	112	100	27
67574	Vincristine	39	80	> 135	83	46
36354	TMCA	100	NT††	60	NT	0
3096	Demecolcine	10	85	50	16	93
82151	Daunorubicin	58	90	127	NT	NT
26980	Mitomycin C	40	NT	150	172	81
3053	Actinomycin D	45	65	> 175	20	90
24559	Mithramycin	13	36	122	0	70

*Treatment ip, qd, Day 1 to death.
†Treatment ip, qd, Days 3–6; killed on Day 7.
‡Treatment ip, qd, Days 1–10.
§Treatment ip, qd, Days 1–5 or 1–8.
¶Treatment ip, qd, Days 1–11; killed on Day 12.
**Underscored values indicate antitumor activity.
††NT = not tested.

A program decision was then made to discontinue use of the W256 system until the clinical usefulness of such agents, selected for their activity solely on the basis of W256 activity, had been evaluated. L1210 leukemia thus became the routine screen for all materials. Many studies, including that by Goldin et al (13), had indicated that the L1210 system was useful for predicting the clinical effectiveness of a new antitumor agent. However, its resistance to therapy was a disadvantage in using it to select crude natural products for further fractionation and isolation studies; therefore, the P388 lymphocytic leukemia system became the primary screening system for crude natural products (14). This system, with testing procedures almost identical to L1210, is still being utilized for natural products (15). A comparison of the antitumor activity of some plant-derived antitumor agents against various experimental tumor systems is shown in tables 2–4 which contain recent published data by Venditti (16).

Current program planning has led to the evaluation of tumor systems which hopefully will more efficiently uncover clinically useful drugs for treating solid tumors. Two such solid tumor systems, the B16 melanoma (implanted either ip or sc) and the Lewis lung carcinoma (implanted sc or iv), have already been introduced for this purpose (17). Other solid tumor systems are being tested for their usefulness as a secondary screen in evaluating the potential clinical utility of new compounds. This secondary evaluation, called "stage III" screening in our current protocol, is shown in figure 1.

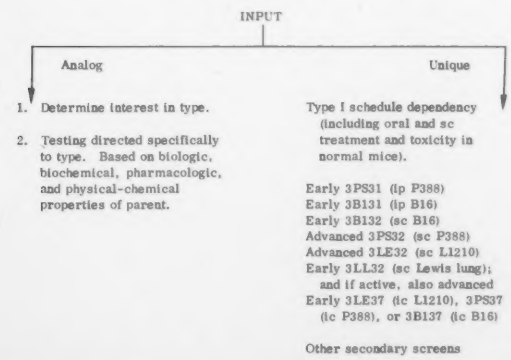
INPUT
Analog
Unique
1. Determine interest in type.
2. Testing directed specifically to type. Based on biologic, biochemical, pharmacologic, and physical-chemical properties of parent.
Type I schedule dependency (including oral and sc treatment and toxicity in normal mice).
Early 3PS31 (ip P388)
Early 3B131 (ip B16)
Early 3B132 (sc B16)
Advanced 3PS32 (sc P388)
Advanced 3LE32 (sc L1210)
Early 3LL32 (sc Lewis lung); and if active, also advanced
Early 3LE37 (ic L1210), 3PS37 (ic P388), or 3B137 (ic B16)
Other secondary screens

FIGURE 1.—Stage III screen for synthetics and natural product isolates.

REFERENCES

1. LEITER J, BOURKE AR, SCHEPARTZ SA, ET AL. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. VI. Plant extracts. Cancer Res [suppl] 21 (Part 2, No. 3):93–98, 1961.
2. MILLER WT, JR. Impact upon screeners of the front-end redesign. Cancer Chemother Rep Part 2, vol 5(1):253–263, 1975.
3. EAGLE H, and FOLEY GE. The cytotoxic action of carcinolytic agents in tissue culture. Am J Med 21:739–749, 1956.
4. ———. Cytotoxicity in human cell cultures as a primary screen for the detection of antitumor agents. Cancer Res 18:1017–1025, 1958.
5. FOLEY GE, EAGLE H, SNELL EE, ET AL. Studies on the use of in vitro procedures for the screening of potential antitumor agents: comparison of activity in mammalian cell cultures and microbiological assays alone and in combination with experimental antitumor activity. Ann NY Acad Sci 76:952–960, 1958.

6. SMITH CG, LUMMIS WL, and GRADY JE. An improved tissue culture assay. I. Methodology and cytotoxicity of anti-tumor agents. Cancer Res 19:843–846, 1959.
7. ———. An improved tissue culture assay. II. Cytotoxicity studies with antibiotics, chemicals, and solvents. Cancer Res 19:847–859, 1959.
8. KUPCHAN SM, BAXTER RL, ZIEGLER MF, ET AL. Podolide, a new antileukemic norditerpene dilactone from *Podocarpus gracilior*. Experientia 31:137–138, 1975.
9. KUPCHAN SM, SWEENY JG, BAXTER RL, ET AL. Letter: Gnididin, gniditrin, and gnidicin, novel potent antileukemic diterpenoid esters from *Gnidia lamprantha*. J Am Chem Soc 97:672–673, 1975.
10. FOLEY GE, MCCARTHY RE, BINNS VM, ET AL. A comparative study of the use of microorganisms in the screening of potential antitumor agents. Ann NY Acad Sci 76:413–441, 1958.
11. THAYER PS, GORDON HL, and MACDONALD M. In vitro growth inhibition by 3837 compounds tested for antitumor activity: comparison of tumor cell culture and microbial assays. Cancer Chemother Rep Part 2, vol 2(1):27–53, 1971.
12. Cancer Chemotherapy National Service Center. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep 25:1–66, 1962.
13. GOLDIN A, SERPICK AA, and MANTEL N. A commentary. Experimental screening procedures and clinical predictability value. Cancer Chemother Rep 50:173–218, 1966.
14. VENDITTI JM, and ABBOTT BJ. Studies on oncolytic agents from natural sources. Correlations of activity against animal tumors and clinical effectiveness. Lloydia 30:332–348, 1967.
15. GERAN RI, GREENBERG NH, MACDONALD MM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). Cancer Chemother Rep Part 3, vol 3(2):1–103, 1972.
16. VENDITTI JM. Relevance of transplantable animal-tumor systems to the selection of new agents for clinical trial. *In* Pharmacological Basis of Cancer Chemotherapy. Baltimore, The Williams and Wilkins Co, 1975, pp 245–270.
17. GERAN RI, GREENBERG NH, MACDONALD MM, ET AL. Modified protocol for the testing of new synthetics in the L1210 lymphoid leukemia murine model in the DR&DP, DCT, NCI. Cancer Chemother Rep Part 2, vol 5(1):15–19, 1975.

Isolation and Chemical Characterization of Antitumor Agents From Plants[1]

Monroe E. Wall,* M. C. Wani, and Harold Taylor[2]

SUMMARY

This paper reviews methods which have evolved at the Research Triangle Institute during the last 15 years for the isolation and characterization of antitumor agents from plants. The isolation procedures stress mild, nonchemical methods. Solvent partition and the Craig Counter Current Distribution are used during the early stages of fractionation. Subsequent purification involves many types of chromatography: adsorption, partition, thin-layer, preparative thin-layer, gel-exclusion, and medium- and high-pressure liquid chromatography. The pure compound is crystallized from a suitable solvent. The fractionation is monitored with in vitro and in vivo bioassays. Physical methods used for structure determination are: ultraviolet, infrared, and nuclear magnetic resonance spectrometry, and X-ray crystallography.

[Cancer Treat Rep 60:1011–1030, 1976]

FRACTIONATION TO ISOLATE ACTIVE AGENTS

Fractionation is guided in most cases by in vivo bioassays of antitumor activity in mice (1), primarily with P388 leukemia, although L1210 leukemia is also used when appropriate. The test data shown in the tables in this paper are expressed as mg/kg of extract or pure compound required to produce the specified increase in lifespan (ILS). An ILS of 25% is the *minimal* accepted *positive* response; a positive response is defined as the dose (mg/kg) which gives a Test/Control (T/C) value of 125%. An attempt is made to correlate in vivo activity with in vitro bioassays. The most frequently used in vitro bioassay is the 9KB cytotoxicity assay. Data from this assay are expressed as the ED50, defined as the concentration (μg/ml) of sample which will produce a 50% reduction in cell growth. A value of 20 μg (2×10^1) is the minimal positive response. When P388 or L1210 activity is correlated with 9KB activity, fractionation can proceed at a much more rapid rate and at considerably less cost. In some cases, when in vivo activity is correlated with growth inhibition of a microorganism, a microbiologic system can be conveniently used to guide fractionation.

Fractionation is initiated with simple methods which result in minimal risk of destroying or altering active antitumor principles. With one exception, no chemical reaction of any kind is utilized during the concentration and isolation process. The one exception is acid-base treatment when the active agent is an alkaloid that will not be affected by this treatment. Simple solvent partition methods are used as early as possible to remove a maximal quantity of inactive constituents. If reasonably good activity is not obtained in an early stage of fractionation, further work with the plant sample is discontinued since activity rarely improves significantly as fractionation progresses.

The six-digit numbers with a "B" prefix that follow plant names in the text, figures, and tables were assigned by the NCI to the original active extracts identified by primary screening. This number is used throughout fractionation and individual fractions are identified by three-digit suffixes preceded by "F." For example, the chloroform fraction F125 in figure 1 is completely identified as *Camptotheca acuminata* (B654996 F125). Similarly, when a

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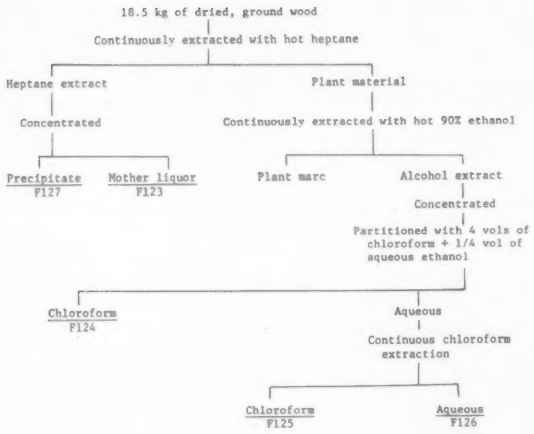


NSC No.	Weight (g)	9KB	Best % T/C in L1210 leukemia
F123	468	5×10^{1}	<125 at 200 mg/kg
F124	222		209 at 50 mg/kg
F125	32	5×10^{-1}	214 at 50 mg/kg
F126		9×10^{1}	128 at 200 mg/kg
F127	10	2×10^{1}	<125 at 200 mg/kg

FIGURE 1.—Preliminary fractionation of *Camptotheca acuminata* (B654996).

pure compound is isolated it is identified by a suffix preceded by "K," eg, K141 in table 6.

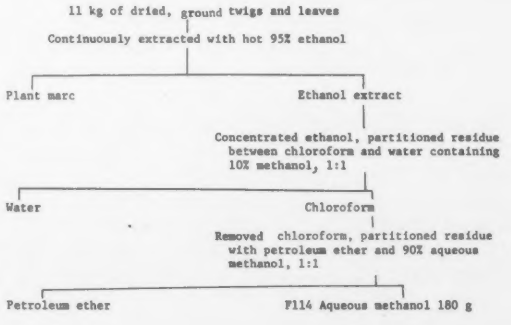
General Extraction and Initial Solvent Partition

Initial large-scale extractions, ie, ≥ 10 kg of dry plant material, were previously carried out with hexane or hexane/heptane followed by 95% ethanol. While this procedure is satisfactory on a small scale, the use of large quantities of hexane in kettles or extractors is both time consuming and potentially dangerous because of the flammability of this solvent. Now, the initial extraction is conducted with 95% ethanol followed by concentration, and then partition of the aqueous residue between water and chloroform. Concentration of the chloroform fraction and partition of the residual solids between 90% methanol/water and hexane completes the initial fractionation. The active constituent of most plant materials is adequately extracted with 95% ethanol. With but few exceptions, it is present in the chloroform fraction and in the 90% methanol/water fraction after the partition of the chloroform-soluble substances between aqueous methanol/hexane. It is advisable to perform an initial small-scale solvent extraction with about 500 g of dry plant material and determine by 9KB cytotoxicity and P388 leukemia bioassays in which fraction(s) activity is concentrated. Antitumor activity is concentrated in the methanol/water fraction except with plants that contain active water-soluble glycosides, tannins, saponins, and the like (2).

Figure 1 illustrates the former solvent extraction procedure used during the isolation of camptothecin (3) (NSC-94600) from *Camptotheca acuminata* (B654996). This is a rare example in which fractionation was guided by L1210 bioassay (1). There was excellent correlation between the 9KB in vitro assay and the L1210 leukemia in vivo assay. The plant material was first extracted with heptane; the residual plant marc was then extracted with ethanol. The ethanol solubles were partitioned between chloroform and 5% aqueous ethanol. The aqueous fraction was partitioned between chloroform and water. The chloroform fraction was highly active and there was virtually no active component in the water or heptane fractions.

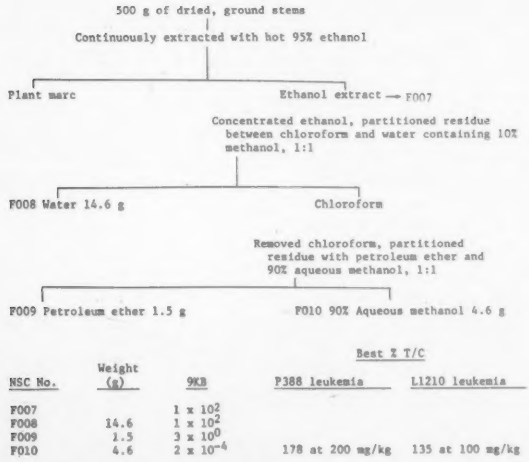
Other examples are offered in figures 2 and 3 which respectively illustrate large-scale fractionation of *Colubrina texensis* (B632355) and preliminary fractionation of *Hernandia cordigera* (B645817). In each case the activity against P388 leukemia was concentrated in the methanol/water phase. The T/C values for this phase are > 170. In each example illustrated, 9KB cytotoxicity is correlated with increased survival in P388 leukemia. In the aqueous or petroleum ether phases, cytotoxicity was either absent or much lower, and there was no P388 leukemia activity.

When cytotoxicity is of reasonable magnitude ($\leq 1 \times 10^{1}$), further fractionation can be continued with plant samples as small as 200–500 g. Usually, extracts which are active in 9KB are also active in P388 leukemia. In some cases, however, plant extracts are inactive in 9KB and active in P388, but


11 kg of dried, ground twigs and leaves
Continuously extracted with hot 95% ethanol
Plant marc
Ethanol extract
Concentrated ethanol, partitioned residue between chloroform and water containing 10% methanol, 1:1
Water
Chloroform
Removed chloroform, partitioned residue with petroleum ether and 90% aqueous methanol, 1:1
Petroleum ether
F114 Aqueous methanol 180 g

NSC No.	Weight (g)	9KB	Best % T/C in P388 leukemia
F114	180	3×10^{-2}	195 at 16 mg/kg

FIGURE 2.—Large-scale fractionation of *Colubrina texensis* (B632355).


500 g of dried, ground stems
Continuously extracted with hot 95% ethanol
Plant marc
Ethanol extract → F007
Concentrated ethanol, partitioned residue between chloroform and water containing 10% methanol, 1:1
F008 Water 14.6 g
Chloroform
Removed chloroform, partitioned residue with petroleum ether and 90% aqueous methanol, 1:1
F009 Petroleum ether 1.5 g
F010 90% Aqueous methanol 4.6 g

NSC No.	Weight (g)	9KB	Best % T/C P388 leukemia	L1210 leukemia
F007		1×10^2		
F008	14.6	1×10^2		
F009	1.5	3×10^0		
F010	4.6	2×10^{-6}	178 at 200 mg/kg	135 at 100 mg/kg

FIGURE 3.—Preliminary fractionation of *Hernandia cordigera* (B645817).

the P388 activity is of a rather low order with a T/C value of < 150.

Figure 4 illustrates an exceptional fractionation method used for the isolation of alkaloids from *Fagara macrophylla* (B670942). The literature indicated that alkaloids were the likely active constituents of species of *Fagara*. The plant extract gave a strong positive alkaloid test so a procedure was used designed specifically for alkaloids—the liberation of the free alkaloids, initially present as salts of plant acids, by ammonia.

Counter Current Distribution (CCD)

The dried residue after the initial solvent partition is a mixture. Although much inactive material is removed by solvent partition, this procedure does not concentrate the active agent. Having started with 10 kg of plant material, at this stage there may be ≥ 0.2 kg of residual solids. If only half of this can

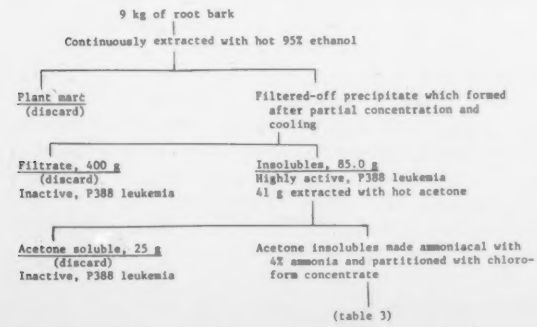

9 kg of root bark
Continuously extracted with hot 95% ethanol
Plant marc
(discard)
Filtered-off precipitate which formed after partial concentration and cooling
Filtrate, 400 g
(discard)
Inactive, P388 leukemia
Insolubles, 85.0 g
Highly active, P388 leukemia
41 g extracted with hot acetone
Acetone soluble, 25 g
(discard)
Inactive, P388 leukemia
Acetone insolubles made ammoniacal with 4% ammonia and partitioned with chloroform concentrate
(table 3)

FIGURE 4.—Isolation of *Fagara macrophylla* (B670942) alkaloids.

be removed prior to chromatography, subsequent purification will be much more efficient.

One of the best methods to achieve this is the Craig CCD, developed by the late Lyman C. Craig of the Rockefeller Institute, New York, NY. This method relies on the different partition constants for each of the substances in a mixture between two immiscible liquids (4). If enough mixing chambers (tubes) are utilized, the compounds can be separated into different chambers along a normal curve based on their individual partition constants. The chief objective of CCD is to obtain a reasonably uniform distribution of solute in all tubes. The apparatus used ranges from simple separatory funnels to elaborate automatic equipment. Many immiscible solvent systems can be used.

A serious problem is encountered in the application of the Craig CCD because most of the plant antitumor substances which have been isolated are extremely water insoluble. Hence, a judicious mixture of several organic solvents, some of which are water immiscible and some of which are water miscible, must be utilized with an aqueous phase in order to dissolve the active agent in both the upper and lower phases. A number of standard solvent systems for water-insoluble compounds have been developed. These can readily be modified to suit a particular case. In practice, the appropriate solvent systems are first developed on a small scale.

Table 1 provides an example of CCD designed for large-scale separation. In this example 300 g of a crude extract of *Arnica chamissonis* (B631957) was being purified. The solvent system was chloroform-carbon tetrachloride-methanol-water (15:35:40:10). The upper phase (1200 ml) of this equilibrated solvent system (predominantly methanol-water) was added to each of the 11, 4-liter separatory funnels. The crude plant extract (300 g) was then dissolved in 1200 ml of the lower phase (predominantly chloroform-carbon tetrachloride) and added to the first funnel, and the liquids were equilibrated by shaking. The lower phase was withdrawn and transferred to the second funnel. An equal volume of the fresh lower phase was added to the first funnel. Both mixtures were equilibrated by shaking, and the lower phase from the second funnel was added to the third, that from the first was added to the second, and the fresh lower phase was added to the first. The procedure was repeated until the lower phase had moved to the last funnel. The freeze-dried residue from each funnel was tested as one sample. A fairly uniform weight distribution was obtained. Again, in vitro cytotoxicity was correlated with in vivo leukemia activity. The active constituent in the original 300 g of crude plant extract was concentrated in the active F061-F062 fractions. Fi-

TABLE 1.—Craig CCD of active principles of *Arnica chamissonis* (B631957)*

NSC No.	Tube	Weight (g)	9KB	Best % T/C in P388 leukemia
Starting material	—	—	3×10^{-2}	
F056	0	31.5	4×10^{1}	Inactive at 80 mg/kg
F057	1	20.2	1×10^{0}	
F058	2	18.2	6×10^{-1}	130 at 80 mg/kg
F059	3	15.0	4×10^{-1}	
F060	4	16.5	5×10^{-1}	170 at 80 mg/kg
F061	5	19.0	2×10^{-2}	
F062†	6	25.0	3×10^{-2}	160 at 20 mg/kg
F063	7	43.5	2×10^{-1}	
F064	8	39.5	3×10^{-1}	150 at 40 mg/kg
F065	9	52.5	3×10^{0}	
F066	10	52.0	2×10^{-1}	145 at 80 mg/kg

*300 g of crude aqueous methanol extract was subjected to 11 tube CCD. 1200-ml phases of chloroform, carbon tetrachloride, methanol, and water (15:35:40:10) were shaken in 4-liter separatory funnels.

†Chromatographed on Sephadex LH20 and silica gel to produce helenaline acetate K090, T/C in P388 = 165% at a dose of 8 mg/kg.

TABLE 2.—Craig CCD of crude *Camptotheca acuminata* extract*

Tube	Weight (g)	9KB	Best % T/C in L1210 leukemia
Starting material	—	8×10^{0}	172 at 200 mg/kg
0	10.9	$> 1 \times 10^{2}$	Inactive at 200 mg/kg
1	3.7	$> 1 \times 10^{2}$	Inactive at 200 mg/kg
2	1.2	—	—
3,4	1.5	$> 1 \times 10^{2}$	Inactive at 200 mg/kg
5	0.6	—	—
6–8	1.6	1×10^{2}	Inactive at 200 mg/kg
9–11	1.3	5.5×10^{1}	Inactive at 200 mg/kg
12–15	1.3	3.4×10^{1}	Inactive at 200 mg/kg
16–18	1.3	4×10^{0}	150 at 200 mg/kg
19	0.6	—	—
20,21	1.6	2×10^{-1}	155 at 100 mg/kg
22	0.9	—	—
23	1.1	$< 1 \times 10^{0}$	155 at 50 mg/kg
24	1.4	—	—
25	1.6	$< 1 \times 10^{0}$	201 at 50 mg/kg
26	1.5	—	—
27	2.3	6×10^{-1}	156 at 50 mg/kg
28,29	0.5	—	—

*40 g of chloroform extract was subjected to 30 tube Craig CCD using *t*-butanol, benzene, and water (12:10:14).

nal purification of the active compound, helenalin acetate (NSC-166124), by chromatography was greatly facilitated.

Table 2 shows another example of CCD, used here for fractionation of the residue from the crude chloroform extract of *Camptotheca acuminata* (F124, F125, fig 1). The active constituents in 40 g of the crude chloroform fractions were concentrated in tubes 22–27, yielding about 9 g of solids which contained almost all the active materials. There was good correlation between the 9KB cytotoxicity and the in vivo L1210 leukemia activity. Pure camptothecin is active in the same T/C range at a concentration of about 0.5–1.0 mg/kg. Thus, the concentrates contained between 1% and 2% of the active constituent. These crude fractions would normally be further purified by chromatography, but, when the solvents were partially concentrated, crude camptothecin of about 75%–80% purity precipitated and was easily purified by further crystallization.

Chromatography

Pure active compounds are isolated by chromatog-

TABLE 3.—Chromatography of crude *Fagara macrophylla* (B670942) alkaloid fractions

Residue from chloroform extract treated with 75% methanol and 25% chloroform; crystals formed

Crystals, 1.4 g
9-Methoxydihydronitidine
Active in P388 leukemia

8.0 g
Active in P388 leukemia

Chromatography (8 g) of mother liquor on 1300 g of Sephadex LH20 in methanol/chloroform (3:1)

Fraction No.		Weight (mg)	Best % T/C in P388 leukemia
1–48		651	Inactive at 4 mg/kg
49–52	crystals (nitidine chloride)	54	181 at 4 mg/kg
49–52	mother liquors	149	170 at 4 mg/kg
53–55		72	
56–62	crystals	1217	Inactive at 4 mg/kg
56–62	mother liquors (oxynitidine)	377	
63–65		76	
66–68		43	135 at 2 mg/kg
69–73		127	
74–86	crystals (9-methoxydihydronitidine)	1860	204 at 8 mg/kg
74–86	mother liquors	930	

raphy. The simplest system is adsorption chromatography with gravity flow columns ½–4 inches in diameter, the latter almost of pilot plant size. The columns are packed with adsorbents like silica gel, Florisil, or alumina. Semipurified active fractions dissolved in a solvent are added to the top of the column. Components of the solution pass through the column at differing rates depending on their individual adsorption coefficients and are eluted into an automatic fraction collector.

The collected fractions are examined by thin-layer chromatography (TLC). Fractions with similar TLC patterns are combined and monitored by standard in vitro or in vivo bioassays.

Sephadex LH-20 is a cross-linked resin used for separating macromolecules. In theory, it separates on a molecular-weight basis. In practice, however, there is also some type of adsorptive process involved because substances with similar molecular weights but with different chemical structures can also be separated.

Table 3 illustrates a purification achieved by only one pass through Sephadex LH-20. This is unusual and was successful because a large quantity of the active constituent was in the crude alkaloid fraction. The active chloroform fraction (cf, fig 4) from *Fagara macrophylla* (B670942) was concentrated, and a crystalline compound, 9-methoxydihydronitidine (NSC-146396), was obtained (5). The mother liquor was chromatographed on Sephadex LH-20 yielding three crystalline compounds, two of which were active. Nitidine chloride was eluted in fractions 49–52. Then a considerable amount of inactive crystalline material, later identified as oxynitidine, was obtained in fractions 56–62. Finally, additional crystalline 9-methoxydihydronitidine was obtained from fractions 74–86 which was highly active in P388 leukemia.

Active constituents are often present in small, sometimes trace, quantities. Tables 4–6 illustrate the complex isolation of an active agent from *Colubrina texensis* (B632355) (6). The active methanol-water fraction (cf, fig 2) was chromatographed on Florisil in a large column (table 4). Activity was concentrated in fraction F123, which contained many "cuts" from the fraction collector. This fraction was chromatographed on Sephadex LH-20 (cf, table 5). Activity, monitored with the 9KB bioassay, was further concentrated. The most active fraction was F130. Further chromatography on silica gel followed by preparative thin-layer chromatography on silica-gel and alumina, and finally another chromatography on Sephadex LH-20, was required to obtain the pure, active compound (table 6). The pure crystalline product, K141, in table 6 was colubrinol. The yield of colubrinol was 0.00005%. This isolation was possible only because of the remarkable sensitivity of the in vitro and in vivo bioassays.

Partition Chromatography

Partition chromatography can also be used effectively, especially when the active compound has properties (eg, acidic or basic) which permit use of a

TABLE 4.—Purification of *Colubrina texensis* (B632355) by chromatography*

NSC No.	100-ml fractions	Weight (g)	9KB	Best % T/C in P388 leukemia
Starting material	—	—	3×10^{-2}	195 at 16 mg/kg
F115	1–64	12.0	2×10^{1}	
F116	65–139	1.7	2.1×10^{1}	
F117	140–164	5.9	2.9×10^{1}	
F118	165–234	0.5	2.4×10^{1}	
F119	235–270	9.5	2.7×10^{0}	
F120	271–310	8.9	5×10^{-3}	
F121	311–335	1.5	4×10^{-1}	
F122	336–352	1.9	3×10^{-3}	
F123	353–394	3.9	1×10^{-4}	213 at 2 mg/kg
F124	395–432	5.8	2×10^{-3}	
F125	433–493	9.0	3×10^{-1}	
F126	9 liters	36.0	1.55×10^{1}	

*The methanol/water fraction F114 from fig 2 was dried onto 1 kg of Florisil and chromatographed on 3 kg of Florisil. The column was eluted by increasing the concentration of methanol in chloroform by 5% every 4000 ml. The active material was eluted between 35% and 50% methanol. Fractions were not started until 8000 ml had been eluted.

TABLE 5.—Purification of *Colubrina texensis* (B632355) by chromatography*

NSC No.	20-ml fractions	Weight (mg)	9KB
Starting material	—	—	2×10^{-3}
F127	3–9	73	3.3×10^{1}
F128	10–12	342	4×10^{-1}
F129	13	137	2×10^{-3}
F130	14–15	204	3×10^{-4}
F131	16	108	2×10^{-3}
F132	17–19	376	2×10^{-2}
F133	20–21	295	5.5×10^{0}
F134	22–23	290	2.7×10^{1}
F135	24–25	135	4.8×10^{1}
F136	26–28	76	$> 1 \times 10^{1}$
F137	29–33	44	5.8×10^{0}
F138	34–42	22	1.9×10^{0}
F139	Methanol insoluble	2200	3.6×10^{1}

*From 3.8 g of F123, the most active fraction from table 4, 2.2 g of methanol-insoluble material was removed. The remainder of methanol-soluble mother liquor was chromatographed on 200 g of Sephadex LH20 in methanol. Fractions were started after 200 ml had been eluted.

TABLE 6.—Final purification of *Colubrina texensis* (B632355) by chromatography*

NSC No.	Weight (mg)	9KB	Best % T/C in P388 leukemia
K141	10	1×10^{-6}	Toxic from 0.32 to 0.04 mg/kg 154 at 0.02 mg/kg 177 at 0.02 mg/kg 177 at 0.01 mg/kg 195 at 0.005 mg/kg

*Fraction F130 (tubes 14 and 15) weighing 204 mg (table 5) was rechromatographed on 40 g of silica gel. A gradient from benzene to methanol was used. The residue (154 mg) from tubes 53–86 was rechromatographed twice on silica gel ChromAR 1000 sheets using benzene/acetone (8:2). The quenching zone was eluted with chloroform/methanol (9:1) and rechromatographed on an alumina preparative thin-layer plate using chloroform/methanol (8:2). This material weighing 15 mg was eluted from 2 g of Sephadex LH20 in methanol and gave 10 mg of a white powder.

TABLE 7.—Partition chromatography of alkaloids of *Cephalotaxus harringtonia* (B619154)*

NSC No.	Fractions	Weight (g)	Dragendorff spots	Gas chromatograph: Major peaks (cm)	Gas chromatograph: Minor peaks (cm)	Best % T/C in P388 leukemia
F041	27–33	0.09	1+	Not done		146 at 4 mg/kg
	34–70	1.93	2+	Not done		
F042	71–165	0.83	1+	Not done		175 at 4 mg/kg
	166–298	0.32	1+	11.0	6.5, 9.0	
K043 (methoxyharringtonine)	299–350	0.20	1+	11.0		>214 at 0.5 mg/kg
	351–400	0.17	1+	11.0	6.5, 8.0, 9.0	
	401–556	0.27	1+	11.0, 8.0	4.2, 5.7, 7.0	
	557–689	0.27	1+	8.0	11.0	
K044 (harringtonine)	690–935	0.36	1+	8.0		203 at 0.5 mg/kg
	936–996	0.08	1+			

*A semipurified alkaloid fraction (6.7 g) was dissolved in benzene and filtered. The benzene-soluble material weighing 5.7 g was chromatographed on a 1000-g cellulose partition column. The mobile phase was benzene and the stationary phase was pH 6.4 phosphate buffer.

buffer at an appropriate pH for the stationary phase. Very effective separation can occur as the mobile phase carries the compound through many partitions in the column. An example is the isolation of some of the active principles of *Cephalotaxus harringtonia*. The structures of these compounds have been determined by Powell et al (7), and are described elsewhere in this issue. Table 7 illustrates partition chromatography of *Cephalotaxus harringtonia* alkaloids in our laboratory. A semipurified alkaloid fraction was chromatographed on a cellulose partition column with phosphate buffer, pH 6.4. Combined fractions were monitored by the Dragendorff alkaloid test on TLC and more effectively by gas-liquid chromatography (GLC). Two crystalline compounds, K043 (methoxyharringtonine) and K044 (harringtonine) (NSC-124147), were isolated. Each had a characteristic retention time by GLC. The pure compounds were well separated by the partition column from each other and also from other minor alkaloids present.

High-Pressure Liquid Chromatography (HPLC)

GLC cannot be used in the isolation of many plant antitumor agents because their molecular weights are too high or too many polar functional groups are present. However, HPLC is generally useful both for monitoring and preparative purposes. Columns with materials of very fine particle size are used. They may be of analytic size, ≤ 1/8 inch in diameter, or much larger, 1/4–1/2 inch in diameter, for preparative purposes. Pressure > 5000 pounds per square inch is developed by modern HPLC instruments. Instruments are available that will yield programmed gradient elution of any type desired.

Detectors can be used with HPLC that measure refractive index, ultraviolet absorption, or fluores-

FIGURE 5.—Separation of camptotheca alkaloids by HPLC. Mixture of camptothecin (A), methoxycamptothecin (B), and hydroxycamptothecin (C). Mobile-phase system: solvent A = 0.5% MeOH in 40% isooctane/$CHCl_3$; solvent B = 5.0% MeOH in 40% isooctane/$CHCl_3$. Concave gradient (curve No. 10, Waters Associates model 660 solvent programmer): 0–100% B in A. Flow rate: 4.0 ml/min. Chart: 0.5"/min. Detector: 254 nm UV at 0.32 attenuation.

cence of compounds eluted. Refractive index detectors are relatively nonspecific and are not particularly sensitive, but are useful with many kinds of substances. Ultraviolet (UV) and fluorescence detectors are very sensitive and are useful when isolating substances that absorb UV rays or are fluorescent.

When a mixture of similar substances is injected into an HPLC column, they move through the adsorbent at variable rates and will have been separated by the time they pass through the detector. A UV detector measures the UV absorption of each constituent. The absorption, which is proportional to concentration, is recorded on a graph which shows peaks representing the elution of each constituent.

Figure 5 shows the application of HPLC for monitoring the isolation of hydroxycamptothecin from a mixture also containing camptothecin and methoxycamptothecin (8). A value, expressed here in inches (it can also be expressed as retention time), is obtained for each pure compound. With this information, the procedure can be scaled up with appropriate columns to isolate a pure compound. The columns can be packed with pure adsorptive substances like specialized types of silica gel or gel-exclusion materials. Partition chromatography can also be performed under high pressure. Usually the stationary phase is polar, but reverse partition can be used in which the stationary phase is nonpolar. Many column-packing materials are now available.

Isolation of an antitumor agent from a crude extract of *Streptosporangium* species (B163423) was accomplished with preparative-pressure chromatography using less expensive equipment which is easily within the means of almost any laboratory.

Table 8 shows the conventional chromatography of the crude extract on silicic acid. A large number of fractions were combined according to similarity in TLC patterns and monitored in three bioassay systems. Many fractions inhibited growth of *B. subtilis* and were active in 9KB cell culture and in vivo P388 leukemia. Three of the less polar fractions, F792–F794, were highly active in all three systems, but TLC showed them to be a complex mixture. Fractions F795–F797 were also active in *B. subtilis* and P388 leukemia but less active in 9KB. These were primarily one spot on TLC and therefore were combined and rechromatographed on a preparative medium-pressure column. This heavy-wall glass column will withstand pressures ≤ 100 pounds. It is used with a Chromatronix Model CMP-3 pump which will not generate higher pressures.

The adsorbent was finely ground silicic acid. Silica gel (TLC grade) can also be used. Moderate pressure must be applied because the adsorbents are much

TABLE 8.—Chromatographic purification of antitumor constituents of *Streptosporangium sp.* (B163423)*

NSC No.	30-ml fractions	Weight (mg)	*B. subtilis*, 5 μg (mm)	9KB	Eluting solvent	Best % T/C in P388 leukemia
F766	1–8	359	0	$> 1 \times 10^{-1}$	C/A + 1% MeOH	Inactive
F767	9–14	267	0	$> 1 \times 10^{-1}$		
F777	15–17	163	0	$> 1 \times 10^{-1}$	↓	160 at 0.2 mg/kg
F778	18–28	363	0	$> 1 \times 10^{-1}$		
F779	29–49	329	7	$> 1 \times 10^{-1}$	3% MeOH	151 at 0.2 mg/kg
F780	50–53	263	0	$> 1 \times 10^{-1}$	↓	
F781	54–60	139	7	$> 1 \times 10^{-1}$		169 at 0.2 mg/kg
F782	61–84	101	9	$> 1 \times 10^{-1}$	7% MeOH	163 at 0.1 mg/kg
F783	85–92	113	9	$> 1 \times 10^{-1}$	↓	
F784	93–106	72	9	$> 1 \times 10^{-1}$		178 at 0.1 mg/kg
F785	107–113	71	8	$> 1 \times 10^{-1}$	10% MeOH	
F786	114–118	100	9	$> 1 \times 10^{-1}$	↓	160 at 0.2 mg/kg
F787	119–132	133	11	1.6×10^{-1}		181 at 0.2 mg/kg
F788	133–138	121	11	$> 1 \times 10^{-1}$	10% MeOH	
F789	139–174	812	11	$> 1 \times 10^{-1}$	15% MeOH	185 at 0.05 mg/kg
F790	175–212	1175	11	$> 1 \times 10^{-1}$	↓	196 at 0.2 mg/kg
F791	213–264	1306	12	1.7×10^{-2}		196 at 0.1 mg/kg
F792	265–312	1230	16	1.4×10^{-2}	↓	
F793	313–336	346	16	1.4×10^{-2}		Toxic at 0.05 mg/kg
F794	337–352	132	15	1.2×10^{-2}	15% MeOH	
F795†	353–380	196	16	$> 1 \times 10^{-1}$	↓	181 at 0.05 mg/kg
F796†	381–412	123	15	$> 1 \times 10^{-1}$		
F797†	413–470	226	14	1.9×10^{-2}	25% MeOH	187 at 0.05 mg/kg
F798	471–505	121	12	$> 1 \times 10^{-1}$	↓	
F799	506–600	433	9	$> 1 \times 10^{-1}$	50% MeOH	187 at 0.1 mg/kg
F800	S.M.		12	1.3×10^{-2}		232 at 0.1 mg/kg

*12 g of a semipurified fraction received from Bristol Laboratories was rechromatographed on 1 kg of silicic acid. The eluting solvent was chloroform/acetone (C/A) (4:1) with increasing amounts of methanol.

†Combined for further chromatography.

TABLE 9.—Isolation of carminomycin I from crude mixture of antitumor constituents*

NSC No.	10-ml fractions	Weight (mg)	*B. subtilis* (5 μg)	9KB	Eluting solvent	Best % T/C in P388 leukemia
F833	1–16	46.3	0	$> 1 \times 10^{-1}$	Chloroform + acetone 4:1	
F834	17–21	18.5	12	1×10^{-2}	+ 10% MeOH	
F835	22–75	60.1	15	6.3×10^{-4}		
F836	76–83	8.6	23	$< 1 \times 10^{-5}$	20% MeOH	231 at 0.2 mg/kg
F837	84–94	15.5	23	$< 1 \times 10^{-5}$		
F838	95–132	36.8	21			
K839†	133–153	157.1	21	8.7×10^{-3}	30% MeOH	211 at 0.2 mg/kg
F840	154–190	45.0	15	$> 1 \times 10^{-1}$		
F841	191–204	14.1	17	5.5×10^{-2}		
F842	205–239	48.0	12	$> 1 \times 10^{-1}$	100% MeOH	182 at 0.1 mg/kg

*Fractions F795, 796, and 797 were combined and rechromatographed on 150 g of silicic acid 100 mesh from Mallinckrodt. The 3/4-inch heavy wall glass column was packed with chloroform-acetone (4:1) and 10% methanol and eluted with increasing amounts of methanol under pressure.

†Pure carminomycin I.

too fine to permit gravity flow. The fractions were collected in a fraction collector and combined after monitoring with TLC. Several fractions (table 9) were highly active against *B. subtilis*, KB cell culture, and P388 leukemia. The major fraction (K839) is identical to carminomycin I (NSC-277184) (9).

To summarize, plant fractionators now have at their disposal a broad galaxy of methods for the purification of plant antitumor agents. With sensitive in vitro assays which often are correlated with in vivo activity, fractionation can be rapidly guided to obtain active compounds, present at times at concentrations as low as 0.00005% (6).

CHARACTERIZATION OF PLANT ANTITUMOR AGENTS

This section will describe the techniques used in the characterization of pure plant antitumor agents. A number of general physical measurements are routinely made for each new compound including melting point (mp) (Kofler mp microscope), the specific optical rotation, and an elemental analysis. The purity of the compound is verified by analytic TLC or by HPLC or GLC when feasible.

Spectroscopic Methods

Spectra of unknown compounds are interpreted by comparing them with spectra of compounds of known chemical structures. UV absorption spectra provide information about conjugated chromophores present in a compound (10); infrared (IR) absorption spectra show the presence of hydroxyl, carbonyl, and many other functional groups (11).

Nuclear magnetic resonance (NMR) spectroscopy is one of the most useful methods available for structure determination. With one type, proton magnetic resonance (PMR) (12), spectra show the environment of protons in a molecule. The other type, ^{13}C NMR spectroscopy (CMR), is a new technique (13) based on the presence of the very small amount of isotopic ^{13}C present in all carbon compounds. The minute amounts of ^{13}C present produce very weak signals which can be detected only with a computer using Fourier transform techniques. Initially, rather large samples were required which made the method impractical for many natural products. A probe which requires less than a milligram of compound is now being developed in collaboration with the JEOL Co. Whereas the complex splitting is characteristic of PMR spectra, CMR spectra are simple showing a single line for each carbon atom present. There are a good many analogies to PMR spectra; eg, aromatic carbon or unsaturated carbon atoms are always found downfield from the carbons which are aliphatic and which do not have neighboring carbonyl or hydroxyl groups.

High-resolution mass spectrometry (14) provides the precise molecular weight of compounds; for many it may provide more accurate data than elemental analysis since it is less affected by impurities. Useful information can also be obtained from the low-resolution fragmentation pattern of a compound.

X-ray crystallography is the most recently developed and most useful method of structure determination (15). It requires crystals of suitable size and shape, the preparation of which can be more time consuming than the crystallographic analysis. When X-rays pass through a crystal a certain amount of scattering occurs and the regular alignment of elements produces a characteristic pattern of spots that can be detected with a photographic plate or scintillation counter. The structure of the compound is determined from the scattering pattern.

In practice, a single crystal is mounted on a goniometer head and several photographs are taken

from different angles with Weissenberg and/or precession cameras. From these photographs the dimensions of the unit cell and the space group of the crystal are determined. The spots are indexed according to the crystal plane which gives rise to them, and their relative intensities are measured. Earlier, the intensities were estimated visually or with a densitometer. Now an automatic diffractometer that uses a scintillation or proportional counter is employed. Intensity data are then processed with a digital computer to derive the structure.

At one time it was necessary to prepare a heavy atom (eg, bromine) derivative of a compound. Such derivatives are no longer necessary for structure determination.

Chemical Procedures

Usually there is insufficient compound available for extensive degradative studies, so simple chemical methods are used. Qualitative tests (eg, for alkaloids, cardiac glycosides, tannins, flavonoids, etc) provide information about the nature of the molecule by color reactions. Reactions like hydrolysis of glycosidic linkages permit the identification of the aglycone and the sugar more readily. Esterification or de-esterification and methylation or demethylation are other simple reactions which are frequently used. Derivatization of hydroxylic or amino functions as esters or amides increases solubility for IR or NMR studies. Halogenated derivatives are formed for X-ray crystallography. Subsequent sections will provide examples of the methods used.

The applications of these techniques will vary from the compound which is totally unknown to the compound about which much can be anticipated from literature describing related substances.

Structure of Camptothecin

Determination of the camptothecin structure (3) required the application of many of the procedures mentioned in the preceding paragraphs. Figure 6

Camptothecin

Light yellow needles, mp 264°C-267°C (dec)

$[\alpha]_D^{25}$ +31.3°

M^+ 348.1117; calculated for $C_{20}H_{16}N_2O_4$: 348.1111

Camptothecin acetate

Mp 271°C-274°C (dec)

M^+ 390.1217; calculated for $C_{22}H_{18}N_2O_5$: 390.1216

FIGURE 6.—Physical properties of camptothecin and its acetate.

gives some of the analytic data for camptothecin and its monoacetate. The high mp suggested a possible aromatic cyclic-ring structure. Some initial qualitative reactions indicated that the compound was not phenolic and gave negative indole, Dragendorff, and Mayer tests even though it contained nitrogen. This meant that the nitrogen could not be very basic. It could not be induced to form crystalline salts, could not be methylated, and did not react with bicarbonate or carbonate which indicated the absence of carboxyl. It was quantitatively converted to sodium salt with sodium hydroxide, suggesting the presence of a lactone.

Figure 7 shows the IR spectrum of camptothecin and figure 8 interprets this spectrum. Thus, from the IR spectra a hydroxyl, a lactone, and possibly a lactam or conjugated ketone were suspected. A conjugated ketone was ruled out because the band at 1660 cm^{-1} was not affected by sodium borohydride reduction. The IR spectra also indicated the presence of aromatic linkages. This is essentially confirmed by the UV spectra (fig 9) interpreted in table 10. This indicates a stable aromatic system with extended conjugation. Since no change appears in the presence of sodium hydroxide or sodium borohydride, phenolic hydroxyl was ruled out because UV absorption would be greatly altered in the presence of base. PMR was obtained with the monochloroacetate derivative in deuteriochloroform solution because camptothecin was too insoluble in this solvent.

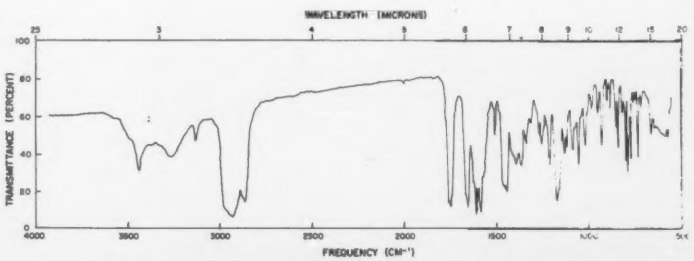


FIGURE 7.—IR spectrum of camptothecin *Nujol Mull.*

Camptothecin:
3440, 1760-1745, 1660, 1610, 1585 cm^{-1}

Camptothecin acetate:
1770-1748, 1245 cm^{-1}

Chief functional groups:

-OH (probably 1 hydroxyl which is nonphenolic. Location of ester band at 1748-1770 suggests $-C(=O)-C-O-C(=O)-CH_3$ moiety.)

$-C(=O)-O-$ (lactone. IR of Na salt shows absence of any bands between 1800 and 1700 and presence of broad carboxylate band at 1620-1585.)

$-N-C(=O)-$ (lactam? 1660 cm^{-1} suggests either lactam or conjugated ketone; latter ruled out because band is unaffected by $NaBH_4$ reduction.)

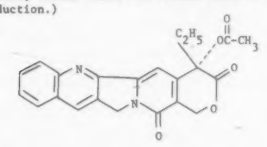


FIGURE 8.—Interpretation of IR spectrum of camptothecin.

The NMR spectrum of the chloroacetate in deuteriochloroform illustrated in figure 10 and its interpretation in table 11 demonstrate an ethyl group in camptothecin. Clearly shown are the two protons in ring E containing the lactone moiety, the two protons in ring C which appear further downfield and which are benzylic to the aromatic ring B, and the aromatic protons in rings A and B.

At this point, much information about camptothecin had been obtained and a variety of reasonable but not necessarily correct structures could be postulated. X-ray crystallography provided the complete structure and stereochemistry. It might be questioned whether all the other data were necessary since the X-ray crystallographer alone could have defined the structure. However, the additional data combined with the X-ray studies enables one to

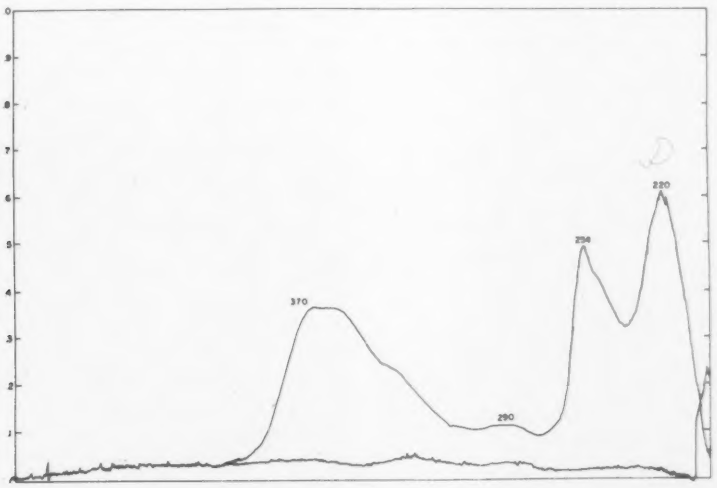


FIGURE 9.—UV spectrum of camptothecin in 95% ethanol.

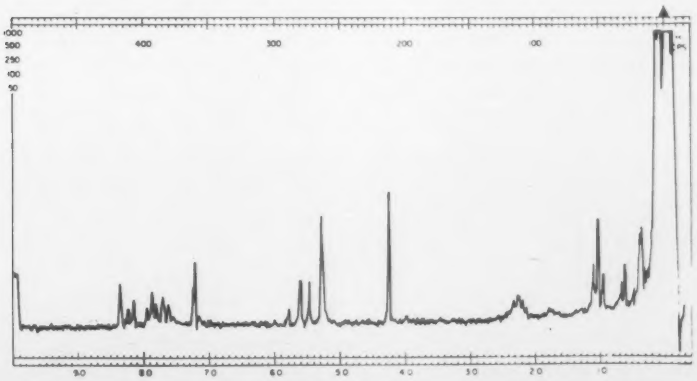


FIGURE 10.—PMR spectrum of camptothecin chloroacetate, 100 MHz, $CDCl_3$.

TABLE 10.—Principal bands in the UV spectrum of camptothecin

λ max	ε
220	37,320
254	29,230
290	4,980
370	19,900

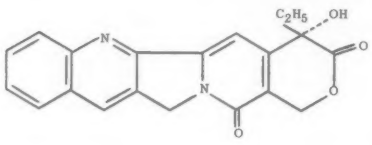
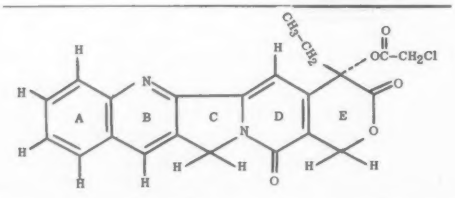


TABLE 11.—Interpretation of NMR spectrum of camptothecin chloroacetate

Chemical shift (δ)	No. of protons (multiplicity)	Assignment
1.08	3 (triplet)	CH_2CH_3
2.26	2 (multiplet)	$-O-\overset{O}{\overset{\|}{C}}-CH_2CH_3$
4.24	2 (singlet)	$ClCH_2C-O-$
5.26	2 (singlet)	$ArCH_2-N-$
5.54	2 (quartet JAB = 17 cps)	$ArCH_2-O-$
7.22–8.36	6	Aromatic protons



proceed much more rapidly and confidently since false leads can be quickly discarded.

Structure of Colubrinol

When the structure of related compounds is known, the elucidation of the structure of an unknown plant antitumor agent, even if very complex, can be completed rapidly, frequently without X-ray crystallography. Figure 11 illustrates the structures of very active ansa macrolides isolated from *Colubrina texensis* and table 12 shows the physical properties of these compounds. Compound *3* is maytanbutine, isolated from *Maytenus ovatus* by Kupchan et al (16). Two new compounds, colubrinol acetate (compound *1*) and colubrinol (compound *2*), were isolated from *Colubrina texensis* (6). It was evident from a publication by the Kupchan group that compounds *1* and *2* were similar to maytanbutine. The quantities were insufficient to obtain crystals suitable for X-ray crystallography.

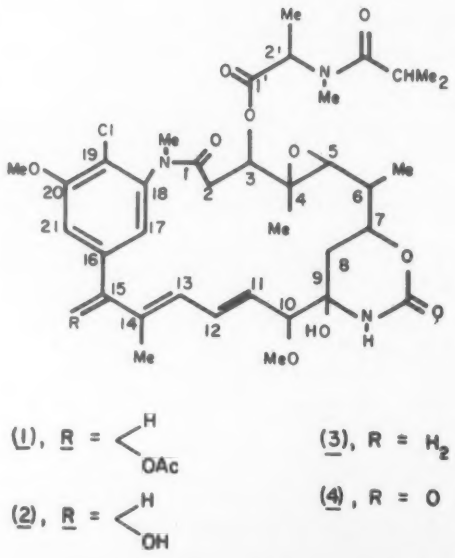


FIGURE 11.—Ansa macrolides from *Colubrina texensis*.

The mass-spectral data in table 12 show the relationship of the *Colubrina* compounds to maytanbutine. Colubrinol does not give a parent ion by electron impact mass spectrometry. It loses H_2O + HNCO in the mass spectrometer and its true molecular weight was 735. This differs by only one oxygen from that of maytanbutine; it is most likely that a hydroxyl has replaced one of the hydrogens in the maytanbutine structure.

Figure 12 compares the IR spectra of this series. The spectra of colubrinol and maytanbutine are

TABLE 12.—Physical properties of ansa macrolides isolated from *Colubrina texensis* (B632355)

Ansa macrolides	Mp (°C)	$[a]_D^{25}$ ($CHCl_3$)	M^+ (high resolution mass spectrometry)	Formula
Colubrinol acetate (*1*)	179–182 (needles)	−127°	777.3249	$C_{39}H_{52}ClN_3O_{12}$ (777.3238)
Colubrinol (*2*)	194–196 (needles)	−94°	674.2929 (M−61, H_2O + HNCO)	$C_{36}H_{50}ClN_3O_{11}$ (674.2967)
Maytanbutine (*3*)	178–180	−126°	719.3191	$C_{38}H_{50}ClN_3O_{10}$ (719.3183)

identical in the carbonyl region, indicating that the carbonyl functions of the two compounds are the same. The IR spectrum of colubrinol acetate is identical except for the peak in the 1735 cm^{-1} region which is now greatly intensified because of the effect of the acetyl carbonyl.

Figures 13–15 compare the PMR spectra of colubrinol, maytanbutine, and colubrinol acetate respectively. Colubrinol and maytanbutine have similar spectra except for the singlet at 5.48 ppm assigned to the C-15 proton of *2*. On acetylation, as shown with colubrinol acetate *3*, this signal is shifted downfield to 7.01 ppm. It seems almost certain that the difference between maytanbutine and colubrinol is the C-15 hydroxyl substituent in the latter. There are few locations in the structure (fig 11) where the hydroxyl group could be placed which would cause so little effect on the IR or NMR spectra. A simple reaction confirmed the location of the hydroxyl moiety at the benzylic C-15 location. Oxidation under mild conditions with manganese dioxide forms the ketone, colubrinone *4*. None of the other possible sites of the hydroxyl are so located that they can be oxidized under these conditions. This information with other spectroscopic evidence presented in the original paper (6) proved the structure of the *Colubrina* compounds.

Structure of Taxol (NSC-125973)

Even though X-ray crystallography has given part of a structure, it may be necessary to conduct a few simple reactions to establish the full structure. Taxol, a complex and highly active antitumor agent,

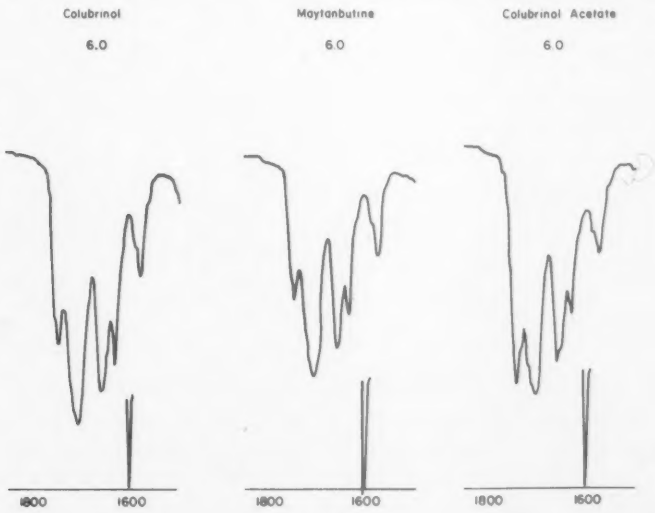


FIGURE 12.—IR spectra (carbonyl region) of ansa macrolides isolated from *Colubrina texensis*, solvent $CHCl_3$.

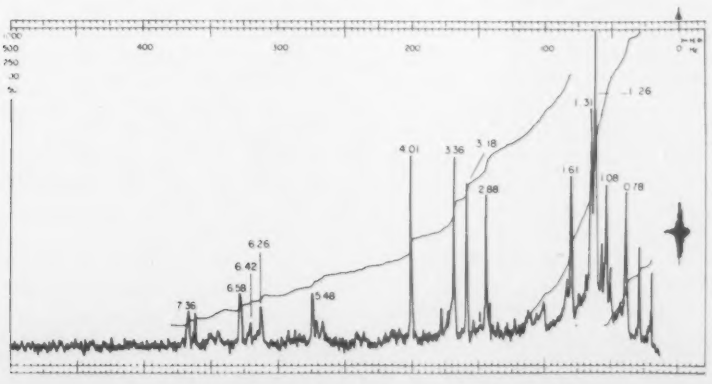


FIGURE 13.—PMR spectrum of colubrinol, 100 MHz, $CDCl_3$.

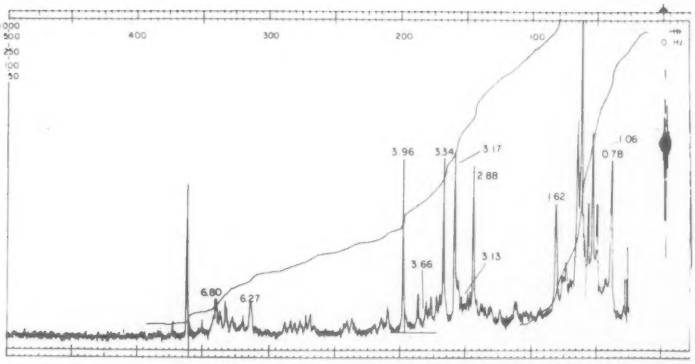


FIGURE 14.—PMR spectrum of maytanbutine, 100 MHz, $CDCl_3$.

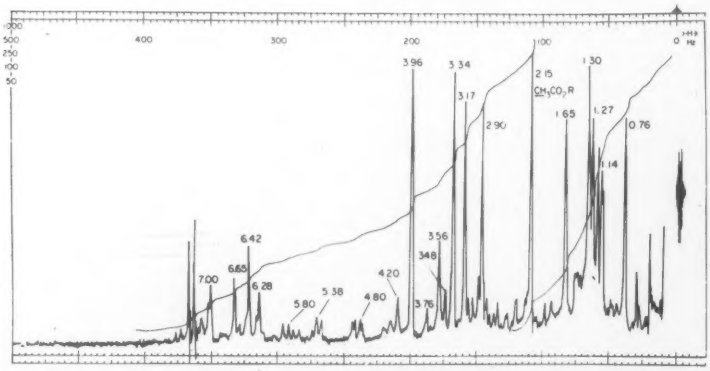


FIGURE 15.—PMR spectrum of colubrinol acetate, 100 MHz, $CDCl_3$.

was isolated from *Taxus brevifolia* (17). Crystals suitable for X-ray crystallography could not be obtained. Preliminary studies indicated taxol consisted of two parts, an α-hydroxy ester and a complex tetraol, which could be obtained after cleavage of the ester by mild methanolysis in the presence of sodium methoxide at very low temperature. The structure of taxol is in the upper half of figure 16. The acetyl group at C-10 is also cleaved by methanolysis. X-ray crystallography of the bis-iodo acetate of the tetraol, compound I in figure 17, showed that the structure of the hydrolyzed taxane moiety was as depicted, with iodoacetyl groups at C-7 and C-10. Crystallography of the parabromobenzoate derivative of the α-hydroxy ester moiety showed its structure to be II as depicted in figure 18. Assuming that no rearrangement of taxol occurs under mild methanolysis, the final structure of taxol requires the placement of the two hydrolyzed ester functions (acetyl and α-hydroxy acyl) on the tetraol.

No reaction occurred when taxol was treated with carefully washed neutral-pH activated manganese dioxide, indicating that the two esters of taxol were located at positions 13 and 10 and the nonallylic hydroxyl groups at 1 and 7 were not esterified in taxol. Oxidation of taxol with manganese dioxide at pH 8 resulted in hydrolysis of one of the esters to yield the conjugated ketone in the bottom half of figure 16. Since a simple conjugated ketone was obtained, the oxidized hydroxyl must be at position 13. If the oxidized hydroxyl group had been at position 10, it would have formed a diketone. Therefore, the acetyl group is at position 10 and the α-hydroxy aromatic ester is at position 13. The presence of the acetyl group at position 10 is further confirmed by the PMR spectrum of the conjugated ketone which shows a singlet at 6.46 ppm; this is in accord with the assigned configuration. Thus, some simple hydrolytic and oxidative reactions helped establish the structure of a very complex substance.

Structure of Holacanthone (NSC-126765)

Intraconversion of an unknown compound to that

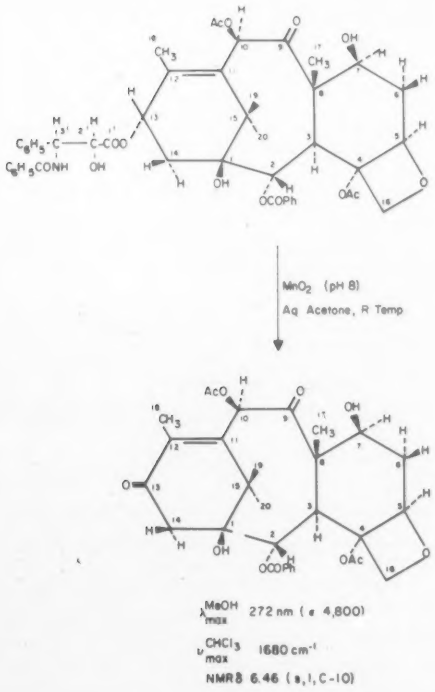


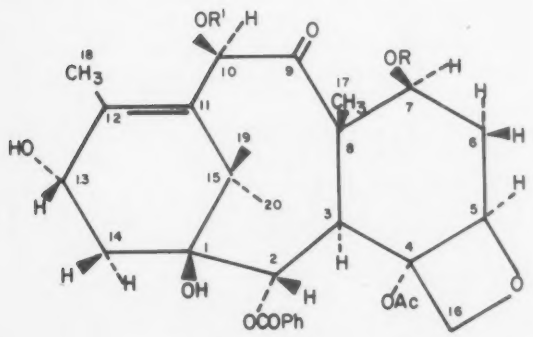
FIGURE 16.—Selective hydrolysis and oxidation of taxol.

of a basic known structure can be used with excellent results in some cases. Holacanthone (fig 19) was isolated from *Holacantha emoryii* (18). This plant is a member of the family Simaroubaceae. Holacanthone has an acetyl group. IR, UV, and PMR spectra indicated similarity in structure to compounds isolated from other species of Simaroubaceae by Polonsky and co-workers (19,20). Figure 20 illustrates a simple intraconversion. Glaucarubinone, a compound of known structure and stereochemistry, was hydrolyzed to yield glaucarubolone.

Holacanthone, which from IR, PMR, and other studies was known to have one acetate ester, was also hydrolyzed to glaucarubolone. The identical tetraacetate was formed from holacanthone and glaucarubolone, confirming the relationship of the two compounds and suggesting that the acetyl ester was most likely located in the same position as the α-hydroxy ester of glaucarubinone. Other studies, particularly PMR studies on holacanthone, showed that the acetyl function was at position 15. Thus, the structure of a complex substance was ascertained, because it was similar to analogous compounds of known structure.

Structure of Nitidine Alkaloids

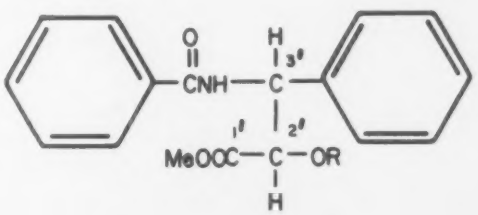
Complete structural elucidation of alkaloids isolated from *Fagara macrophylla* was accomplished by spectroscopic measurements and a few simple chemical reactions (5). Figure 21 shows the interrelationship between nitidine chloride which occurs naturally in the plant, 9-methoxydihydronitidine, an artifact which is produced from nitidine chloride



I, R = R' = H

II, R = R' = ICH_2CO

FIGURE 17.—Structure of alcohol from methanolysis of taxol.



I, R = H

II, R = Br—⟨⟩—CO

FIGURE 18.—Structure of ester from methanolysis of taxol.

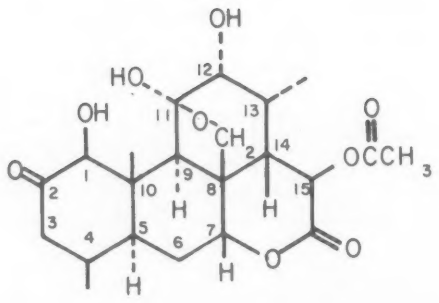


FIGURE 19.—Structure of holacanthone.

(NSC-146397) during isolation, and a known synthetic, 6,7-dimethoxy-2′,3′-methylenedioxy-1,2-benzo-phenanthridine. Nitidine chloride is rapidly converted to 9-methoxydihydronitidine in the presence of methanol and ammonia. In the presence of hydrochloric acid the latter can be converted to nitidine chloride. Both, when heated under sublimation conditions, are converted to the synthetic benzophenanthridine. These simple reactions, plus much UV, IR, and PMR data, completely clarified the structures of these compounds. The artifact, 9-methoxydihydronitidine, is somewhat more active than nitidine chloride.

Structure of Texensine

Mass spectrometry is useful when there is a great amount of mass spectral data available for a class of compounds like the indole alkaloids or when unknown structures consist of fragments for which mass spectral data are available. Texensine, an

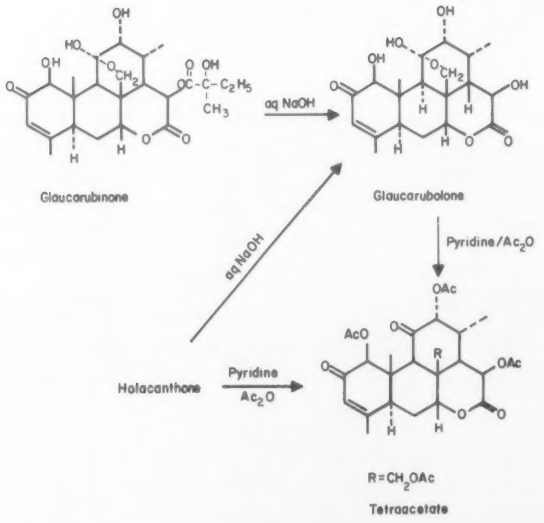


FIGURE 20.—Conversion of holacanthone and glaucarubinone to a common tetraacetate.

inactive compound isolated as a by-product during the fractionation of *Colubrina texensis* (21), is an example of a compound characterized in part by mass spectrometry. *Colubrina* is a genus of the Rhamnaceae; species of this family produce characteristic peptide alkaloids. The mass spectrum of texensine is shown in figure 22. The exact molecular compositions of the various fragments in the mass spectrum of texensine as shown in figure 23 were determined by high-resolution mass spectrometry. With this information and other spectral data (IR, UV, PMR) the structure of texensine was deduced as shown in figure 23.

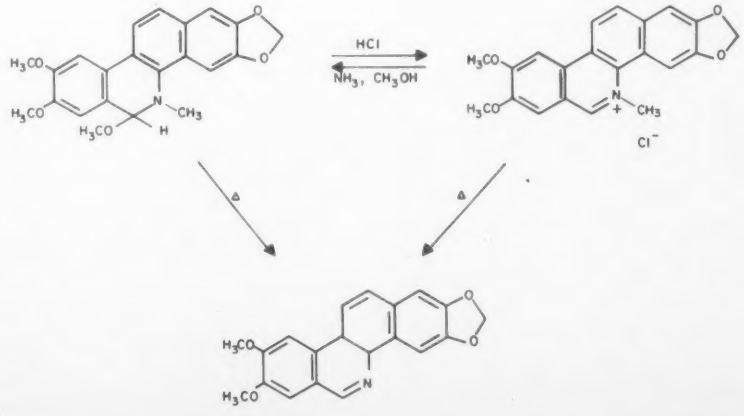


FIGURE 21.—Conversion of 9-methoxydihydronitidine and nitidine chloride to a common intermediate.

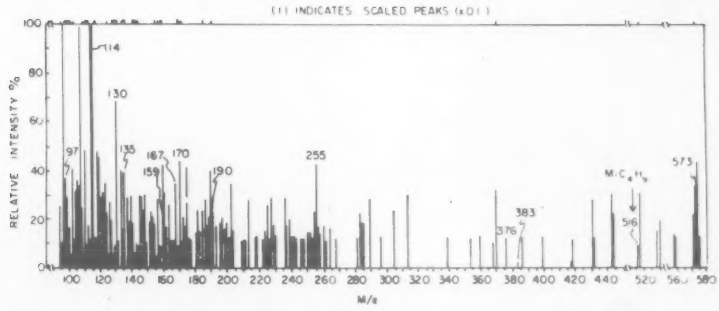


FIGURE 22.—Mass spectrum of texensine.

Structure of Hydroxyquinidine Using ^{13}C NMR

Figure 24 shows CMR spectra for quinidine and a metabolite obtained by hydroxylation of quinidine at the 3 position by liver microsomes (22). The single peak for the 3 carbon shown in the CMR spectra of quinidine disappears and is replaced by a new peak somewhat downfield from the 9 carbon, which also has a hydroxyl substituent. All the other peaks remain constant. Therefore, the new peak has to be due to hydroxylation at the 3 carbon. It is now located downfield similar to the 9 carbon atom.

Structure of Carminomycin I

An antitumor agent (NSC-227184) was isolated from an antibiotic culture of *Streptosporangium* species (23), and recent evidence obtained from the laboratory of Dr. George Pettit, Arizona State University, Tempe, indicates that it is identical to carminomycin I (9). The crystalline compound was obtained as a monohydrochloride. X-ray crystallography of the monohydrate hydrochloride indicated the structure illustrated in figure 25. The properties of NSC-227184 are listed in figure 26. Figure 27 shows the key reaction used to obtain the overall structure. Carminomycin was hydrogenolyzed under very mild conditions to yield the known sugar daunosamine. Acid hydrolysis yielded an aglycone, similar in many respects to a compound described by Russian scientists (9).

Physical properties and structure of the aglycone are described in figure 28. The molecular formula, the absence of any signal for a methoxyl, and the formation of the pentaacetate suggest that the aglycone may be desmethyldaunomycinone. This compound has the same overall structure as the aglycone described by the Russians (9). The X-ray data provided the overall stereochemistry of this amino sugar glycoside and established the stereochemistry of carminomycin I (23).

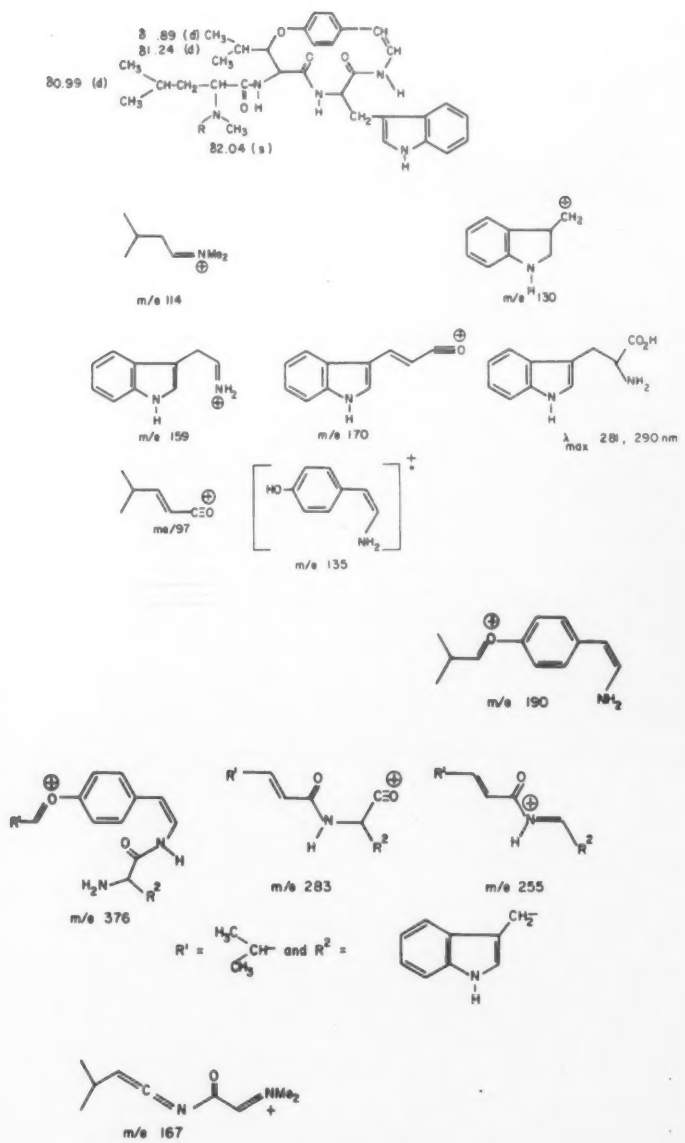


FIGURE 23.—Mass-spectral fragmentation patterns of texensine.

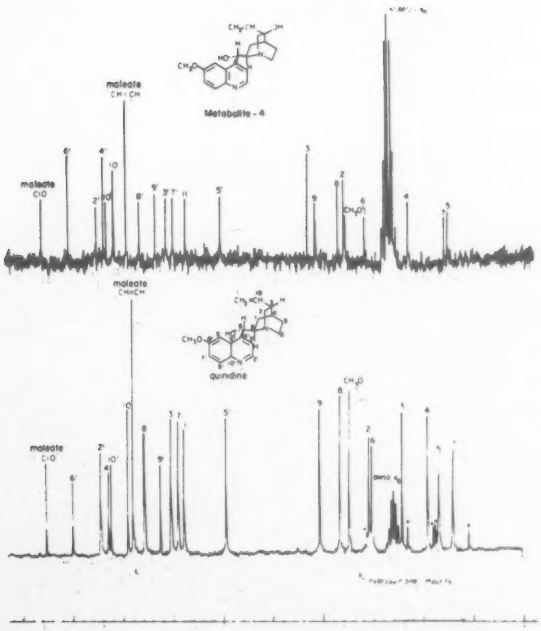


FIGURE 24.—^{13}C NMR spectra of quinidine and a 3-hydroxy metabolite.

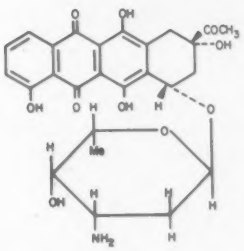


FIGURE 25.—Absolute configuration of carminomycin I.

1. Crystals from $CHCl_3$-acetone-MeOH (4:1:2)
2. Mp 187°C-188°C
3. Molecular formula $C_{26}H_{27}NO_{10}\cdot H_2O\cdot HCl$ (Calculated analysis: C, 54.98; H, 5.32; N, 2.47; Cl, 6.24. Found: C, 55.33; H, 5.32; N, 2.59; Cl, 6.05.)
4. $[\alpha]_D^{25}$ +193° (C 0.15, MeOH)

FIGURE 26.—Physical properties of carminomycin I isolated from *Streptosporangium* species.

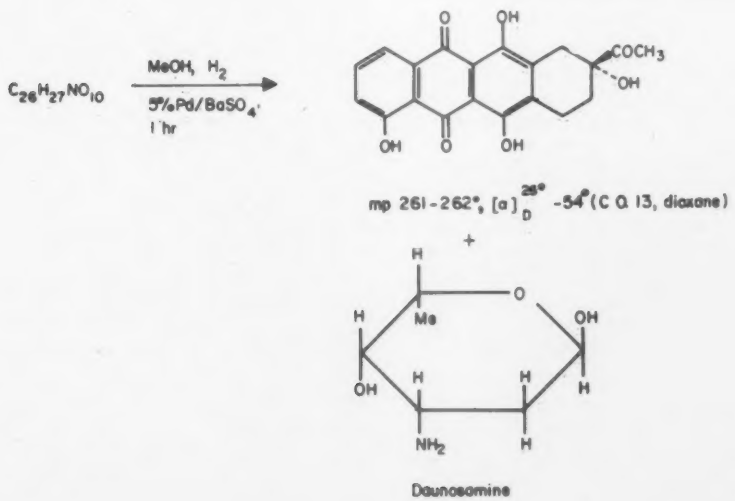
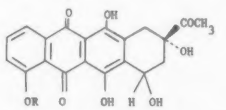


FIGURE 27.—Hydrogenolysis of carminomycin I.

1. Crystals from 13% MeOH/$CHCl_3$-EtOAc
2. Mp 233°C-235°C (dec)
3. $[\alpha]_D^{28}$ +171° (C 0.14, dioxane)
4. Molecular formula $C_{20}H_{16}O_8$ (m/e 384.0845)
5. Forms pentaacetate, $C_{30}H_{26}O_{13}$ (m/e 594.1370; mp 218°C-220°C)
6. NMR spectrum characterized by the absence of a signal for OCH_3



Carminomycinone, R = H
Daunomycinone, R = CH_3

FIGURE 28.—Physical properties and structure of carminomycinone.

REFERENCES

1. GERAN RI, GREENBERG NH, MACDONALD MM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). Cancer Chemother Rep Part 3, vol 3(2):1–103, 1972.
2. WALL ME, TAYLOR H, AMBROSIO L, ET AL. Plant antitumor agents. III. A convenient separation of tannins from other plant constituents. J Pharm Sci 58:839–841, 1969.
3. WALL ME, WANI MC, COOK CE, ET AL. Letter: Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890, 1966.
4. MORRIS CJOR, and MORRIS P. Separation Methods in Biochemistry. New York, John Wiley and Sons, 1964, pp 559–619.
5. WALL ME, WANI MC, and TAYLOR HL. Plant anti-tumor agents. VIII. Isolation and structure of anti-tumor alkaloids from *Fagara macrophylla*. *In* Proceedings of the 3rd International Congress of Heterocyclic Chemistry, Sendai, Japan, Aug 23–27, 1971.
6. WANI MC, TAYLOR HL, and WALL ME. Plant antitumor agents: colubinol acetate and colubrinol, antileukaemic ansa macrolides from *Colubrina texensis*. Chem Commun, 1973, p 390.
7. POWELL RG, WEISLEDER D, SMITH CR, JR, ET AL. Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahedron Lett 11:815–818, 1970.
8. WANI MC, and WALL ME. Plant antitumor agents. II. The structure of two new alkaloids from *Camptotheca acuminata*. J Org Chem 34:1364, 1969.
9. BRAZHNIKOVA MG, ZBARSKY VB, PONOMARENKO VI, ET AL. Physical and chemical characteristics and structure of carminomycin, a new antitumor antibiotic. J Antibiot 27:524–529, 1974.
10. SCOTT AI. Interpretation of the Ultraviolet Spectra of Natural Products. New York, Pergamon Press, 1964.
11. BELLAMY L. The Infrared Spectra of Complex Molecules. New York, John Wiley, 1958.
12. JACKMAN LM, and STEINHELL S. Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry (2nd ed). London, Pergamon Press, 1969.
13. LEVY GC, and NELSON GL. Carbon-13 Nuclear Magnetic Resonance for Organic Chemists. New York, Wiley Interscience, 1972.
14. BUDZIKIEWICZ H, DJERASSI C, and WILLIAMS DH. Structural Elucidation of Natural Products. Alkaloids (vol 1). Steroids, Miscellaneous Classes (vol 2). San Francisco, Holden-Day, Inc, 1964.
15. STOUT GH, and JENSEN LH. X-ray Structure Determination. New York, The Macmillan Co, 1968.
16. KUPCHAN SM, KOMODA Y, COURT WA, ET AL. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J Am Chem Soc 94:1354–1356, 1972.
17. WANI MC, TAYLOR HL, WALL ME, ET AL. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327, 1971.
18. WALL ME, and WANI MC. The isolation and structure of holacanthone, a potent experimental antitumor agent. *In* Proceedings of the 7th International Symposium on Chemistry of Natural Products, Riga, USSR, June 22–29, 1970.
19. POLONSKY J, and BOURGUIGNON-ZYLBER N. Studies of the bitter constituents of the fruits of *Hannoa klaineana* (Simaroubaceae): Chaparrinone and Klainéanone. Bull Soc Chim France 10:2793–2799, 1965.
20. GUADEMER A, and POLONSKY J. Structure of glaucarubinone, a new bitter principle from *Simaruba glauca*. Phytochemistry 4:149, 1965.
21. WANI MC, TAYLOR HL, and WALL ME. Plant antitumor agents. XII. Texensine, a new peptide alkaloid from *Colubrina texensis*. Tetrahedron Lett 47:4675–4678, 1973.
22. HAUSER FM, CHEN T-K, and CARROLL FI. 14-Hydroxycodeinone: an improved synthesis. J Med Chem 17:1117, 1974.
23. WANI MC, TAYLOR HL, WALL ME, ET AL. Letter: Antitumor agents. XIII. Isolation and absolute configuration of carminomycin I from *Streptosporangium* sp. J Am Chem Soc 97:5955–5956, 1975.

Types of Anticancer Agents Isolated From Plants

Jonathan L. Hartwell[1,*]

SUMMARY

The active compounds which have been isolated from plants and tested in the chemotherapy program of the National Cancer Institute since the inception of the plant program (as part of the Cancer Chemotherapy National Service Center) are listed, classified into types, and discussed in terms of their activity in experimental tumor systems. The tumor systems include the most important ones comprising the regular screen at different times and also the slow-growing tumors, B16 melanoma and Lewis lung carcinoma (new). The structure-antitumor activity relationships bring out the desirability for further investigation of certain types of compounds as possibilities for clinical trial. Notes on the current pharmacologic and clinical status of certain compounds are also presented.

[Cancer Treat Rep 60:1031–1067, 1976]

From the earliest days of the Cancer Chemotherapy National Service Center (1956) it was realized that a comprehensive program for testing compounds for antitumor activity must include those of natural as well as synthetic origin. It was also known that nature is able to produce a wide variety of chemical entities of novel structure. Many of the new and novel compounds isolated from natural sources might otherwise have never been discovered, especially those of considerable complexity requiring the development of methods for the creation of new ring systems. Natural products appeared to be a promising source for new types of compounds to test for antitumor activity. Consequently, a fermentation program was initiated in 1956 and in 1957 the plant program followed although a few plant extracts were received and tested in 1956. Procurement of animals and their extracts began in 1962.

Before the establishment of the experimental natural products program, I had conducted a survey of the literature and folklore of plants with reputed efficacy in cancer (1) and found > 3000 different species of such plants reported from all over the world. Previously, I had isolated podophyllotoxin and two other lignans from the mayapple root (*Podophyllum peltatum* L.)(2), all of which were powerfully active against Sarcoma 37 in the mouse; I undertook this survey when I learned that this plant was used by the Penobscot Indians of Maine as a treatment for what was believed to be cancer (3) and that the root of this plant was recommended for cancerous tumors in a book on materia medica and therapeutics published in 1849 (4). It is interesting to observe that several derivatives of podophyllotoxin are now in clinical trial in cancer. Although the literature and folklore can provide leads for plant collecting, it was realized that selective collecting is expensive and that, besides, this kind of input would be insufficient to maintain a large program for a long time. Therefore, a world-wide random collection program was initiated with the expectation not only of uncovering new leads for the isolation of novel compounds but also of ultimately revealing correlations between anticancer activity and botanical classification that would make collection more efficient in terms of finding active agents. The success of these considerations can be judged from this and other papers in this Symposium, as well as by the multitude of published papers already in the literature which arose from our program.

METHODS

The efficient isolation of active compounds from plants in a large program is obviously dependent upon the effectiveness of the extraction procedure

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and the bioassay methods used to detect active plants and to guide the fractionations. I believe it is worth a short discussion of these points before taking up the compounds themselves, because variations in the methods have a profound effect on the types of compounds isolated. Beginning with necessarily arbitrary methods, some improved methods have evolved over the years based on feedback from the results obtained (these are outlined in other papers in this Symposium). For example, the original antitumor screen consisted of the three mouse tumors (Sarcoma 180, Carcinoma 755, and L1210 leukemia) deemed most able to select most of the compounds considered in 1955 to be clinically useful in man. KB cell culture was added in the latter part of 1960. Plant extracts were prepared using different methods and solvents recommended by the suppliers. After it was determined that all the active crude extracts obtained by the different methods could also be uncovered by the use of 50% aqueous ethanol, a standard procedure using this solvent was adopted by all suppliers. In 1966, the Walker 256 rat carcinoma was added to the screen to replace Sarcoma 180, Carcinoma 755, and certain other tumors after an analysis of clinical results (5) showed that 33 of 45 drugs with definite clinical activity could have been predicted by L1210 leukemia and nine of 45 by Walker 256 carcinoma. The newly constituted screen had the disadvantage, from the chemical standpoint, that the ubiquitous tannins and phytosterols were very effective against Walker 256 (as well as Sarcoma 180, Carcinoma 755, and the Lewis lung carcinoma) but not against L1210 leukemia and KB cells; many plants therefore showed activity against Walker 256 and only a very few showed activity against L1210 leukemia and KB cells. Since the tannins and phytosterols could not be developed into useful anticancer drugs (6), a great deal of effort was required to find Walker 256-active agents that were nontannin in nature. The development of a novel procedure for the nondestructive elimination of tannins from plant extracts (7) permitted fractionation of nontannin Walker 256 actives only; these and the KB actives provided the only sources of antitumor plants at this time. Walker 256 was later dropped from the screen pending feedback from the clinic on the usefulness of drugs active in Walker 256 only,

Table 1. -- Activity of agents and of their plants of origin

Agent	Activity	Plant of Origin	Activity
Acer saponin P	WA	Acer negundo	SA
Bruceantin	Bl,LE,PS,KB	Brucea antidysenterica	KB
Camptothecin	LE,PS,WA,KB	Camptotheca acuminata	LE
		Nappia foetida	KB,PS,LE
Cesalin	LL,SA,WA,KB	Caesalpinia gilliesii	SA
3-Desmethylcolchicine	Bl,PS,LE	Colchicum speciosum	KB
Ellipticine	Bl,LE,PS,WA,KB	Bleekeria coccinea	KB,LE,SA
		Ochrosia moorei	KB,CA,LE,SA
Emetine	PS,LE,KB	Cephaelis acuminata	KB
Fagaronine	PS,LE	Fagara zanthoxyloides	PS
Harringtonine	LE,PS,WA,KB	Cephalotaxus harringtonia	KB
Holacanthone	Bl,PS,KB	Holacantha emoryi	KB
Homoharringtonine	LE,PS,KB	Cephalotaxus harringtonia	KB
Indicine N-oxide	Bl,PS,LE,WA	Heliotropium indicum	WA
Lapachol	WA	Stereospermum suaveolens	WA
Maytansine	Bl,LE,PS,KB	Maytenus buchananii	KB
		Putterlickia verrucosa	KB
9-Methoxyellipticine	LE,PS,SA,KB	Bleekeria coccinea	KB,LE,SA
		Ochrosia moorei	KB,CA,LE,SA
Nitidine	LE,PS,KB	Fagara macrophylla	KB
Taxol	Bl,LE,PS,WA,KB	Taxus brevifolia	KB
Thalicarpine	LL,PS,WA,KB	Thalictrum dasycarpum	KB
Tripdioiide	LE,PS,KB	Tripterygium wilfordii	KB
Triptolide	LE,LL(new),PS,KB	Tripterygium wilfordii	KB
Tylocrebrine	CA,LE,PS,KB	Tylophora crebriflora	KB

and the more sensitive mouse P388 leukemia was substituted for L1210 leukemia. The present screen consists of P388 leukemia and KB cells and does not react to the unwanted tannins or phytosterols. In fact, KB cell culture has emerged as the principal predictor of activity for newly isolated compounds against the in vivo tumors that we consider important. This is probably because most antitumor agents are also cytotoxic, and since the KB test is more sensitive than the in vivo tests (eg, P388 and L1210 leukemias), it is able to detect cytotoxic compounds in a complex mixture such as a crude extract or fraction where in vivo activity would be obscured. In practice, a KB-active plant is fractionated until the activity is concentrated into one or a few fractions. The concentrated fractions are then tested against P388 and L1210 leukemia, and activity in one or both tumors is frequently found. A majority of the plant-derived compounds of interest, including those passing the criteria (activity in P388 and L1210 leukemias, B16 melanoma, and the new Lewis lung tumor) for further pharmacologic and clinical study were isolated from plants originally found to be active against KB cells (table 1). The latest development in the preparation of crude plant extracts for testing, which has been described elsewhere in this Symposium, is the preliminary fractionation to eliminate inactive materials and concentrate the active agents present prior to testing. This procedure results in greatly increased yields of plants active in P388 leukemia and KB cells, and produces many high priority plants for fractionation. This development, however, has not yet been in operation long enough to establish its value in producing useful compounds.

RESULTS

The results are summarized in the tables which list the active compounds along with the important tumors against which they have been tested. In many cases, results are available in our files for other less important tumors than the ones listed. The gaps in the data usually stem from a shortage of compound.

The tumor systems considered, with their activity criteria, are listed in table 2. Activity as shown in the "active" columns of the tables does not always reflect reproducible activity. Many materials were tested as suspensions which frequently led to erratic results with compounds of marginal activity. Also, the effort and expense required to obtain additional material precluded the extensive testing of compounds with initial borderline activity. These compounds for which activity has not been "confirmed" have nevertheless been included since our purpose is to show the broad activity relationships of groups of compounds, not to provide the kind of data on individual compounds required for determining whether further pharmacologic testing will be performed.

Table 2. - Tumor systems

Abbreviation	Name; host	Active response
B1	B16 melanocarcinoma; mouse	ILS 1/ ≥40%
CA	Adenocarcinoma 755; mouse	TWI 1/ ≥ 58%
HE	HeLa human carcinoma; cell culture	ED_{50} 1/ ≤1.0
KB	Human epidermoid carcinoma of the nasopharynx; cell culture	ED_{50} ≤1.0 2/
LE	Lymphoid leukemia L-1210; mouse	ILS ≥25%
LL	Lewis lung carcinoma; mouse	TWI ≥58%
LL (new)	" " " "	ILS ≥40%
PS	Lymphocytic leukemia P388; mouse	ILS ≥25% 3/
SA	Sarcoma 180; mouse	TWI ≥58%
WA	Walker carcinosarcoma 256; rat	TWI ≥58%

1. TWI (tumor weight inhibition); ILS (increase in life span); ED_{50}, dose level in μg/ml at which 50% inhibition of growth of cells in vitro is noted vs untreated controls.

2. A few compounds in the following tables have been considered active where the ED_{50} was ≤4.0.

3. In the following tables, activity in PS is expressed by a figure in parenthesis which is the T/C or ratio of average survival of treated animals in days to that of controls x 100. T/C = ILS ÷ 100.

No distinction is made between marginal and high antitumor activity except against P388 leukemia. In this case, the figure in parentheses after "PS" is the highest T/C obtained in any test and does provide a measure of the degree (marginal, high, or intermediate) of activity; this figure is provided because in practice so much depends on the interest generated by the first in vivo tests. While a T/C of 125% is an indication of activity, a figure of 175% is normally required for a priority high enough to warrant advanced screening and preclinical evaluation.

In the tables presented in this paper the source (table 3) of the compounds is acknowledged after the compound name. The classification adopted for the compounds listed therein is given in table 4. Broadly, all of the classes are non-nitrogenous except for the ansa macrolides, the proteins, and the alkaloids; the miscellaneous group is mixed.

Table 3. - Acknowledgments

1. Dr. J. L. Beal	Ohio State University
2. Dr. Jack R. Cole	University of Arizona
3. Commonwealth Scientific and Industrial Research Organization	Melbourne, Australia
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5. Dr. R. W. Doskotch	Ohio State University
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7. Mr. Martin Jacobson	U.S. Department of Agriculture
8. Dr. G. J. Kapadia	Howard University
9. Dr. L. B. Kier	Massachusetts College of Pharmacy
10. Dr. D. G. I. Kingston	Virginia Polytechnic Institute
11. Dr. S. Morris Kupchan	University of Virginia
12. Dr. G. R. Pettit	Arizona State University
13. Charles Pfizer and Co., Inc.	Maywood, N.J.
14. Mr. R. G. Powell	U.S. Department of Agriculture
15. Dr. B. Prescott	National Institute of Allergy and Infectious Diseases
16. Dr. M. Silva	University of Concepcion, Chile
17. Dr. Monroe E. Wall	Research Triangle Institute
18. Dr. E. Gellert	Wollongong University College
19. Dr. J. M. Cassady	Purdue University
20. Dr. Ming-Lu King	National Defense Medical Center, Taiwan
21. Dr. A. C. Casey	Stauffer Chemical Company
22. Dr. D. H. Miles	Mississippi State University
23. Dr. F. C. Chang	University of Tennessee
24. Dr. G. J. Persinos	Bergstrom Toxicology Laboratory

Table 4. - Classification

Tannins
Sterols (incl. simple glycosides; excl. saponins)
Quinones (incl. quinoids and quinols)
Terpenes
Iridoids
Sesquiterpenes
Diterpenes
Tripterpenes (incl. cucurbitacins; excl. saponins)
Lignans
Flavonoids
Saponins and their aglycones
Steroidal
Triterpenoid
Steroid lactones (incl. cardenolides, bufadienolides, withanolides and their aglycones)
Quassinoids (simaroubolides)
Ansa macrolides
Proteins
Alkaloids
Miscellaneous

Tannins

No formal attempts were made to prepare pure compounds from this heterogeneous group of widely occurring polymeric materials after it was shown that the tannin fractions were generally inactive against the predictive tumors (L1210 and P388 leukemias and KB cells), were active against uninteresting tumors (Carcinoma 755, Lewis lung, Sarcoma 180, and Walker 256), and were quite toxic. Moreover, they were generally chemically unstable, being susceptible to air oxidation and further polymerization.

In 1969 (6), 82 different active plant species were listed as owing their activity solely to tannins. Since that time, another 82 species have been similarly identified. Fortunately, the makeup of the current screen precludes the appearance of more plants whose activity is due to tannins.

Sterols (Including Simple Glycosides but Excluding Saponins)

The phytosterols and their glycosides are widely distributed in plants. The ones that have shown antitumor activity are given in table 5. Only daucosterol showed marginal activity in P388 leukemia. Other phytosterols such as cholesterol (NSC-8798), ergosterol (NSC-62791), and stigmasterol (NSC-8095) have been tested in a variety of tumors (6), including Carcinoma 755, L1210 leukemia, Sarcoma 180, and Walker 256, without showing activity; in addition, stigmasterol was inactive in P388 leukemia and ergosterol was inactive in KB cells.

In 1969 (6), 20 different active plants were listed as owing their activity to sterols (mostly β-sitosterol), and since then, another 42 species have appeared. Again, the antitumor screen eliminates most of the plants whose activity is due to sterols.

Quinones (Including Quinoids and Quinols)

Table 6 lists the quinones that have shown antitumor activity. Although some activity has been ob-

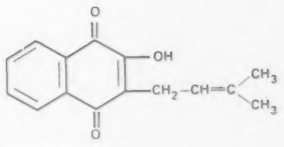
Table 5. - Sterols

	NSC	Active	Inactive
Daucosterol 16/ (β-sitosterol glucoside)	165962	PS(134)	LE, KB
β-Sitosterol	8096	CA, LL, WA	Bl, LE, LL(new) SA, KB
	18173		
	49083		
	86199		

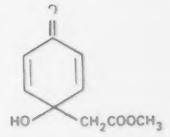
Table 5. -- Quinones

	NSC	Active	Inactive
Alkannin 4/	94524	WA	LE,PS,SA,KB
Alkannin β,β-dimethylacrylate 4/	140377	PS(136),WA	B1,LE
Alkannin monoacetate 4/	140376	WA	
Emodin (aloe-emodin) 11, 16/	38628	PS(127),WA	CA,LE,SA,KB
Jacaranone 6/	251682	PS(165),KB	
Lapachol 13/	11905	WA	B1,CA,LE,LL, LL(new),PS, SA,KB
Taxodione 11/	122419	WA,KB	LE,PS
Taxodone 11/	122420	KB	

QUINONES



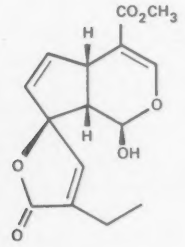
Lapachol
NSC-11905



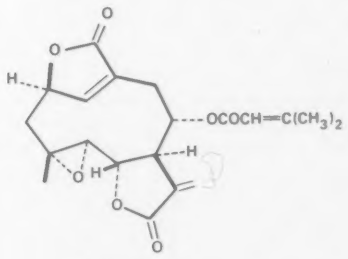
Jacaranone
NSC-251682

FIGURE 1

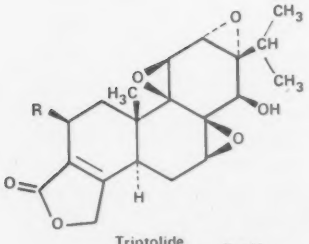
TERPENES



Allamandin
NSC-251690

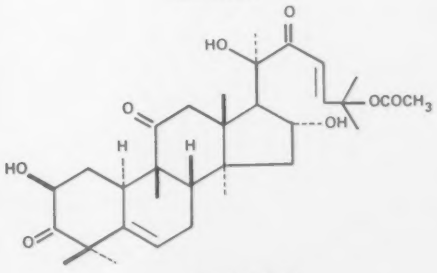


Elephantopin
NSC-100046



Triptolide
NSC-163062 R = H

Tripdiolide
NSC-163063 R = OH



Cucurbitacin B
NSC-49451

FIGURE 2

tained against P388 leukemia (the best is jacaranone),[2] no activity has been found against L1210 leukemia, B16 melanoma, or Lewis lung carcinoma, and this class of compounds cannot presently be considered very promising. Lapachol (fig 1), originally obtained from *Stereospermum suaveolens* (Roxb.) DC., has been carried into clinical trial mostly on the basis of its high Walker 256 activity even when given orally (8). Lack of toxicity permitted large oral doses but sufficiently high blood levels could not be obtained to show a therapeutic effect.

It is perhaps of passing interest that *Tabebuia* and *Tecoma* species, containing lapachol, have long been popularly used in Brazil for cancer and that a *Jacaranda* species, called cancer bush, is popularly used in the Bahamas for skin cancer (1).

[2]Farnsworth NR. Personal communication.

Terpenes

This large and complex group of natural products has proved to be of substantial interest to us. The natural breakdown into chemical subgroups reveals that only the iridoids, sesquiterpenes, diterpenes, and triterpenes have antitumor activity. No activity has been found among the monoterpenes, sesterterpenes, and carotenoids. Figure 2 gives representative examples of the chemical structures of these active classes, namely, an iridoid (allamandin), a sesquiterpene (elephantopin), two diterpenes (triptolide and tripdiolide), and a triterpene (cucurbitacin B). Allamandin was obtained from *Allamanda cathartica* L. (9), elephantopin from *Elephantopus elatus* Bertol. (10), triptolide and tripdiolide from *Tripterygium wilfordii* Hook. fil. (11), and cucurbitacin B from several sources not all Cucurbitaceae—*Cucurbita digitata* Gray,[3] *Luffa graveolens* Roxb.,[4] *Begonia* X *tuberhybrida* Voss (12), and *Datisca glomerata* (Presl) Baill.[5]

a. Iridoids.—Table 7 lists the active iridoids (all of which are lactones) which have been isolated to date. Lack of material has prevented testing in a wider variety of tumors but it is encouraging that at least some P388 leukemia activity has been shown by two of the four substances.

Table 7. - Iridoids

	NSC	Active	Inactive
Allamandicin 11/	251691	PS in vitro	PS,KB
Allamandin 11/	251690	PS(145),KB	
Isoplumericine 11/	112153	PS in vitro	LE
		PS(145),KB	
Plumericine 11/	112152	KB	LE

b. Sesquiterpenes.—Table 8 shows the active sesquiterpenes that have been isolated in the fractionation program. All but one are lactones. Many more have been received and tested in the "synthetic" program but the results obtained do not generally alter the comments made here. A majority (38 of 47) of the compounds have cytotoxic activity (KB or P388 leukemia in vitro), a few (eight of 47) have activity against Walker 256, and a large number (23 of 47) showed activity against in vivo P388 leukemia. A sufficient number of P388 leukemia actives have relatively high T/Cs for some interest to be maintained in this subgroup. Although none has shown activity as yet in L1210 leukemia or in either of the two slow-growing tumors, B16 melanoma and Lewis lung (new), hope remains that additional testing will reveal such activity. There is some evidence that certain chemically reactive functional groups besides the lactone group (α, β-unsaturated carbonyl, epoxy) are important for in vivo antitumor activity and that polyfunctionality seems to increase the chances for activity. However, physico-chemical and steric factors are undoubtedly important also, and the whole question of structure-activity relationships is in need of clarification.

c. Diterpenes.—This subgroup (table 9) has yielded two compounds of high activity in P388 leukemia. Two others, triptolide and tripdiolide, are also active in L1210, and tripdiolide is active against the Lewis lung carcinoma. Additional studies will be performed on the latter two compounds as more plant material becomes available.

d. Triterpenes (excluding saponins).—This group so far has not been promising. The cucurbitacins as a class (table 10) show general cytotoxicity and negative or marginal in vivo antitumor activity against P388 leukemia, L1210 leukemia, B16 melanoma, and Lewis lung tumor (new). Our present screen will continue to identify plants containing

Table 8. - Sesquiterpenes a/

	NSC	Active	Inactive
Ambrosin 2/	85235	PS(180),KB	SA,LE
Arctiopicrine 2/	177853	PS(140),KB	
Artemisiifolin 2/	177852	PS(130),KB	
Baileyin 12/	179192	PS in vitro	
Costunolide 2,5/	106404	KB	LE,WM
Damsin 5/	85249	KB	LE
Elephantin 11/	102817	WA,KB	LE
Elephantopin 11/	100046	WA,PS(160)	LE,LL,SA
10-Epieupatoroxin 11/	135068	KB	
Epitulipinolide 5/	142844	KB	
Erioflorin 11/	144151	PS(127),KB	LE
Erioflorin acetate 16/	251667	PS(131),KB	
Erioflorin methacrylate 16/	251666	KB	PS
Eriolangin 11/	182855	PS(128)	KB
Eriolanin 11/	144152	PS(152),KB	
Eupachlorin 11/	114567	KB	WA
Eupachlorin acetate 11/	114568	WA,PS(155), KB	
Eupachloroxin 11/	114570	KB	
Eupacunin 11/	135020	WA,PS(135), KB	
Eupacunoxin 11/	135021	KB	
Euparotin 11/	104942	KB	
Euparotin acetate 11/	104943	WA, KB	PS
Eupatocunin 11/	135025	KB	
Eupatofolin 11/	135023	PS (150), KB	
Eupatoroxin 11/	114569	KB	
Eupatundin 11/	114566	PS (133), KB	LL, LE, SA, WA

[3]Cole JR. Personal communication.
[4]Dhar ML. Personal communication.
[5]Kupchan SM. Personal communication.

Table 8. - Sesquiterpenes a/ (continued)

	NSC	Active	Inactive
Fastigilin B 12/	176503	PS (137)	Bl, LE, LL(new), KB
Fastigilin C 12/	176507	PS (137)	KB
Gaillardin 11/	106394	KB	LE
Helenalin 12,17/	85236	PS (220), KB	Bl, LE, LL, LL(new), SA, WA
Helenalin acetate 17/	166124	PS (165)	
Isogaillardin 11/	106395	KB	
Liatrin 11/	135034	PS (163)	
Lipiferolide 5/	251676	KB	
Nardol diastereomer (?) b,13/	127084	WA	
Ovatifolin acetate 16/	--	PS (143), KB	
Parthenolide 2/	157035	KB	PS, LE
Paucin c,2/	136722	PS (138)	Bl, LE
Tulipinolide 5/	106405	KB	LE, WA
Vernodalin 11/	124459	WA, KB	
Vernolepin 11/	106398	WA, PS (145), KB	
Vernolide 11/	124460	KB	WA, LE, PS
Vernomenin 11/	116070	PS (136)	KB
Vernomygdin 11/	135072	KB	
Zaluzanin C 2/	177851	PS in vitro	PS
from Acanthospermum 6/	--	PS (147), KB	
" Centaurea 2/	--	PS (150), KB	

a/ All lactones except the one noted.

b/ Not a lactone.

c/ A glycoside.

these compounds because of their KB cell activity. The remaining triterpenes (table 11) which have been isolated are generally not cytotoxic but show activity in Walker 256. Negative or marginal in vivo antitumor activity against the P388 and L1210 leukemias, and against the slow-growing tumors, has made this group of little interest for our program.

Lignans

These are a group of rather uncommon plant constituents which are found in some plants with activity against KB cells. The chemical skeletons of the group are shown in figure 3. The bisbenzocyclooctadiene skeleton on the right is a type recently added to the classic lignan family. Although podophyllotoxin (fig 3) never passed our criteria for pharmacologic and clinical study, several semisynthetic relatives have been in clinical trial here and elsewhere in the world (13). Two of them, VM-26 and VP-16-213, have produced responses in brain tumors, lymphosarcomas, and (in Europe) Hodgkin's

Table 9. - Diterpenes

	NSC	Active	Inactive
Gnidicin 11/	238941	PS (173)	
Gnididin 11/	238942	PS (127)	
Gniditrin 11/	238943	PS (168)	
12-Hydroxydaphnetoxin 11/	239073	PS (131)	
Jatrophone 11/	135037	PS (145), KB	Bl, LE, LL, LL(new)
Mezerein 11/	239072	PS (200)	Bl, LL(new)
Podolide 11/	238978	KB	PS
Taxodione (also under Quinones) 11/	122419	WA, KB	LE, PS
Taxodone " " " 11/	122420	KB	
Tripdiolide 11/	163063	LE, PS (158), KB	Bl, LL(new)
Triptolide 11/	163062	LE, LL(new), PS (131), KB	Bl
Triptonide 11/	165677	KB	LE
from Jatropha	--	PS (141)	

Table 10. - Cucurbitacins

	NSC	Active	Inactive
Cucurbitacin B 2,4,5,11/	49451	PS (135), KB	Bl, CA, LE, LL, SA, WA
" D 5,11,16/	521776	PS (131), KB	LE, WA
" E 11,17/	106399, 521775	LL, KB	Bl, LE, PS, SA, WA
" F 11/	--	WA, KB	LE, PS
" I 11/	521777	KB	LE, PS, WA
" L 2/	112167	PS in vitro, KB	PS, WA
" P 11/	135074	KB	
" Q 11/	135075	KB	
a Cucurbitacin glycoside 11/	--	KB	
" " " 11/	--	KB	
" " " 11/	--	KB	
" " " 11/	--	KB	
Datiscacin (Cucurbitacin R) 11/	144154	KB	
Datiscoside (Cucurbitacin D dehydrospirhamnoside) 11/	144153	LL, PS (150), WA, KB	Bl, LE, LL(new)
Dihydrocucurbitacin B 5,11/	106401	KB	WA
Isocucurbitacin B 11/ (2-Epicucurbitacin B)	106400	KB	

disease. These compounds differ from podophyllotoxin in that they are demethylated at the 4′ position, are epimerized at the 1 position, have a glucoside on the 1-hydroxyl group, and are acetals by reaction with aldehydes. The original 4′-demethylpodophyllotoxin came from *Podophyllum hexandrum* Royle [*P. emodi* Wall. ex Royle]. Table 12 lists the active lignans isolated during fractionation.

While several of the compounds have shown high activity in P388 leukemia, they have not met the other criteria required to justify further study.

Table 11. - Triterpenes

	NSC	Active	Inactive
α-Amyrin 2,4/	114787	WA	Bl, LL(new) PS, KB
Betulin 2/	4644	WA	Bl, CA, LE, LL, SA, WA
Betulinic acid 2,22/	113090	PS (140), WA	KB
Lupeol 2,21/	90487	WA	LE, PS, SA, KB
Ursolic acid 2,6,17/	4060	PS (125)	Bl, LE, LL(new), WA, KB
Uvaol 2/	159627	PS (125)	
from Bursera 2/	--	WA	
" Jatropha 2/	--	PS (158)	
" Maytenus 6/	--	PS (166), KB	
" Rubia 2/	--	WA	

Table 12. - Lignans

	NSC	Active	Inactive
Burseran 2/	123428	WA, KB	
Dehydroanhydropicropodophyllin 2/	--	PS in vitro	
3'-Demethylpodophyllotoxin 6/	251681	PS (130), KB	
Deoxypodophyllotoxin 2,6,11,17,23/	403148	LE, PS (154), KB	Bl, LL, LL(new), SA, WA
5'-Desmethoxy-β-peltatin A methyl ether 2/	126727	PS (189), WA, KB	Bl, LE
(+)-Dimethylisolariciresinol-2α-xyloside 11/	--	KB	PS, WA
Justicidin B 11/	254665	KB	
α-Peltatin 17/	24817, 35463	PS (140), KB LE	Bl, CA, LL, LL(new), SA, WA
β-Peltatin 17/	24819, 35471	PS (168), WA KB	Bl, LE, LL(new), SA
β-Peltatin A methyl ether 2/	126726	PS (172), WA	
Podophyllotoxin 6,11,23/	24818	PS (171), WA KB	Bl, CA, LE, LL, LL(new), SA
Podophyllotoxin glucoside 9/	163024	KB	
Steganacin 11/	172958	PS (140), KB	LE
Steganangin 11/	172956	KB	LE, PS
Steganol 11/	172959	KB	PS
Steganone 11/	172957	KB	PS

Flavonoids

This very common class of plant constituents is generally inactive in our antitumor screen. Many have been submitted as "synthetics." Table 13 lists those showing activity during fractionation. Only one showed marginal activity in P388 leukemia.

Table 13. - Flavonoids

	NSC	Active	Inactive
Centaureidin 11/	106969	KB	PS
Taxifolin 16/	36398	PS (142)	LE, SA
3',5,7-Trihydroxy-3,4'-dimethoxyflavone 11/	106970	KB	PS

Table 14. - Saponins

Steroidal	NSC	Active	Inactive
Myrsine saponin 6,11/	123126	WA	
from Agave 2/	--	WA	
" " 2/	137440	WA	PS
" " 2/	--	WA	
" " 2/	--	WA	
" Hesperaloe 17/	--	WA	
" Solanum 17/	--	WA	
" Trillium 17/	--	MØ	
Triterpenoid			
Acer saponin P 11/	100045	WA	Bl, LE, LL LL(new), PS, KB
" " Q 11/	123429	SA	WA
Celsioside C 4/	173116	WA	LE, KB
from Acer 17/	--	WA, PS (141)	
" Entada 13/	115727	WA, LL	LE, PS, Bl
" Ipomopsis 17/	--	WA	
" Machaeranthera 17/	--	PS (129)	WA
Undetermined			
from Agave 11/	--	LL	
" " 11/	--	SA	
from Agave 17/	--	WA	
" Allium 11/	--	SA	
" Aster 6/	--	WA	PS
" " 17/	--	WA, PS (135)	
from Chrysopsis 11/	--	SA	
" Cyclamen 11/	135029	WA	KB
" Saponaria 13/	77472	WA, SA	CA, LL, LE, PS

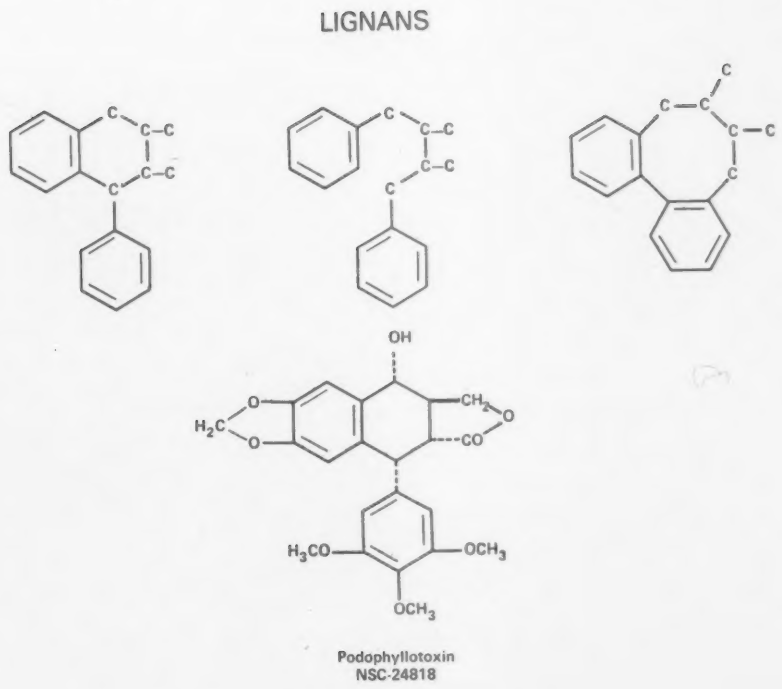
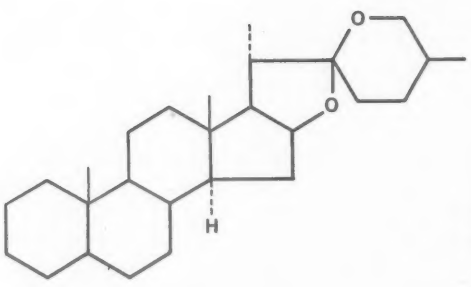


FIGURE 3

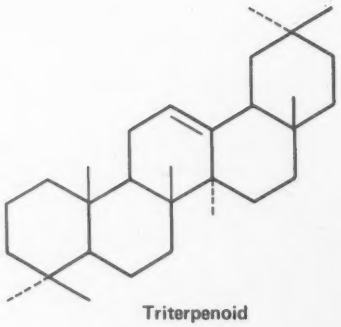
Saponins

This widely disseminated class of plant constituents generally possesses activity against Walker 256 and Sarcoma 180, but lacks activity in KB cells. Twenty-three new active saponins have been added to the ten that were listed in 1969 (6); although isolated in some state of purity, in many cases the nature of the aglycone is undetermined (table 14). They have shown marginal activity against P388 leukemia, and no activity against L1210 leukemia or the slow-growing tumors. Thus, plants whose antitumor activity is attributable to saponins will probably not be detected with our current screen. Nevertheless, since they are so widespread in the plant kingdom, and since many were active in Walker 256, it was decided to continue studies on the best one, Acer saponin P, in order to obtain feedback. Figure 4 gives the carbon skeletons of the two main types of saponins, steroidal and triterpenoid, and the partial formula for Acer saponin P. This substance was obtained from *Acer negundo* L. (14) and is now being prepared in quantities sufficient for pharmacologic and clinical trials.

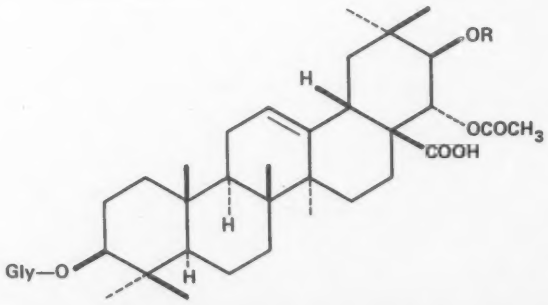
SAPONINS



Steroidal



Triterpenoid



R = Mixture of $-CO(CH\underset{t}{=}CH)_2CH(CH_3)CH_2CH_3$ and

$-CO(CH\underset{c}{=}CH)CH\underset{t}{=}CH)CH(CH_3)CH_2CH_3$

Acer saponin P
NSC-100045

FIGURE 4

Steroid Lactones (Including Cardenolides, Withanolides, Bufodienolides, and Their Aglycones)

The carbon skeletons of these three types of lactones are given in figure 5. The cardenolides, especially, are widely distributed in nature. Table 15 lists the active steroid lactones isolated during fractionation; the type of lactone is indicated in the first column. It is apparent that they are generally cytotoxic with very little activity in any in vivo tumors. Plants containing these compounds will show up in our screen but will be ruled out, for the most part, when KB-active concentrates are subjected to P388 and L1210 leukemia testing.

Quassinoids (Simaroubolides)

This group of bitter plant principles has proved to be of great interest. Figure 6 presents the formulas of the two most promising representatives. Bruceantin was isolated from *Brucea antidysenterica* J.F. Mill. (15), a plant used in Ethiopia and Eritrea for cancer (1), and holacanthone from *Holacantha emoryi* Gray (16). Table 16 outlines data on the active compounds which have been isolated. In general, they are cytotoxic; a large proportion of them are highly active in P388 leukemia and several are also active in L1210 leukemia or B16 melanoma. It is evident that plants containing active quassinoids will be readily detected by our screen. Bruceantin is

Table 15. - Steroid lactones and their aglycones

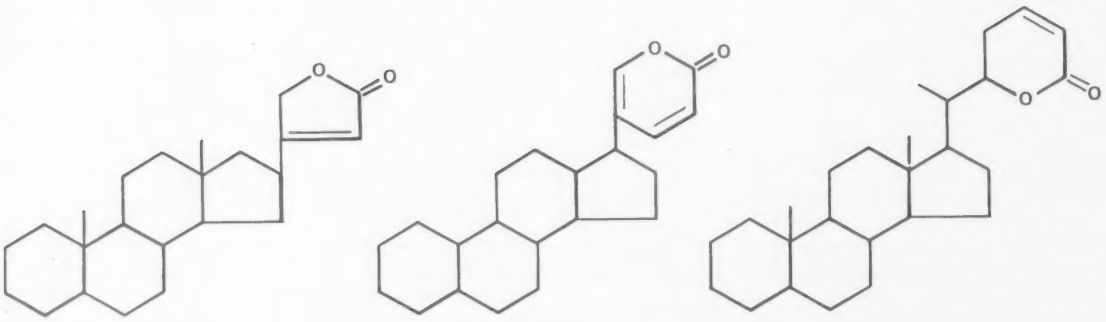
Type a/	Name	NSC	Active	Inactive
C,G	Acobioside A 8/	116788	KB	
C,G	Acolongifloroside K 10/	152149 173717	KB	LE
C,G	Acospectoside A 8/	113569	KB	LE
C,G	Acovenoside A 8,10/	116787	KB	B1, LE, PS
C,G	Acovenoside B 8/	116789	KB	
C,G	Adynerin 2/	251673	KB	B1, LL(new) PS in vitro
C,G	Apocannoside 11/	83216	KB	LE, PS, SA, WA
B,A	Bersaldegenin 3-acetate 11/	135076	WA, KB	
B,A	Bersaldegenin 1,3,5-orthoacetate 11/	135077	KB	
B,A	Bersamagenin 1,3,5-orthoacetate 11/	135032	KB	
B,A	Berscillogenin 11/	--	KB	
B,A	Bersenogenin 11/	251692	KB	
C,G	Calotropin 11/	143925 106393	KB	LE
C,G	Cerberin 2/	251674	PS (130), KB	
C,A	Coroglaucigenin 11/	144150	KB	
C,G	Cymarin 6,11/	7522	KB	CA, LE, LL PS, SA, WA
C,G	Desglucouzarin 6/	--	KB	LE, PS
C,G	Digitoxin 14/	7529	KB	CA, LE, PS, SA, WA
B,A	3-Epiberscillogenin 11/	135067	KB	
C,A	Gitoxigenin 5/	407807	KB	
B,A	Hellebrigenin 3-acetate 11/ (Bufotalidin acetate)	106676	WA, KB	
B,A	Hellebrigenin 3,5-diacetate 11/	109330	KB	WA

Table 15. - Steroid lactones and their aglycones (continued)

Type a/	Name	NSC	Active	Inactive
B,A	16β-Hydroxybersaldegenin 1-acetate 11/	135080	KB	
B,A	16β-Hydroxybersaldegenin 3-acetate 11/	135079	KB	
B,A	16β-Hydroxybersaldegenin 1,3,5-orthoacetate 11/	135078	KB	
B,A	16β-Hydroxybersamagenin 1,3,5-orthoacetate 11/	251695	KB	
C,G	Hyrcanoside 19/	--	PS (133), KB	
C,G	Neriifolin 2/	123976	LL, SA, KB	LE, PS, WA
C,A	Oleandrigenin 5/	148790	KB	LE
C,G	Oleandrin 2/	95089	KB	LE, WA
C,G	Opposide 10/	173716	KB	
C,A	16-Propionylgitoxigenin 5/	160843	KB	
C,G	Rhodexin B 5/	160845	KB	
B,A	Scilliglaucosidin 11/	135036	KB	PS
C,G	Somalin 2/	251698	KB	PS
C,A	Strophanthidin 6/	86078	KB	B1, LE, LL(new), PS
C,A	Uzarigenin 11/	119993	KB	PS, WA
W,A	Withacnistin 11/	135073	KB, WA	
W,A	Withaferin A 11,17/	101088	PS (135), SA WA, KB	
C,G	from Asclepias 6/	--	PS (150), KB	LE
C(?),G	" Crossosoma 2/	--	KB	
--	" Elaeodendron 11/	--	KB	

a/ Symbols: B=bufodienolide, C=cardenolide, W=withanolide, A=aglycone, G=glycoside

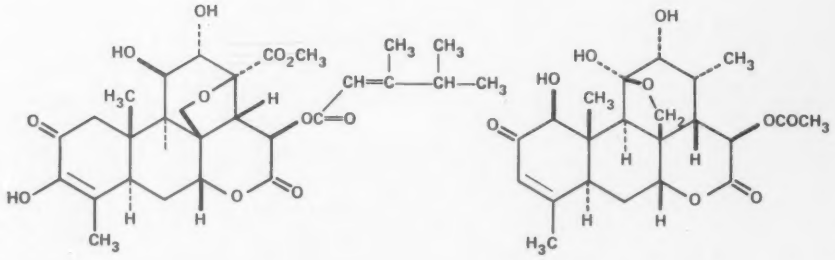
STEROID LACTONES



Cardenolides | Bufadienolides | Withanolides

FIGURE 5

QUASSINOIDS



Bruceantin
NSC-165563

Holacanthone
NSC-126765

FIGURE 6

now being prepared in quantities sufficient for toxicologic and pharmacologic studies.

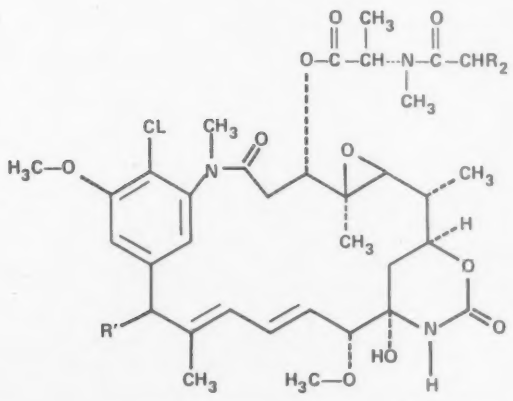
Ansa Macrolides

This is a relatively new class of compounds characterized chemically by the presence of a large macrocyclic lactone ring, frequently *N*-heterocyclic, and incorporating within it an *m*- or *p*-bridged aromatic moiety. The class was originally found among microbial products and the current findings represent their first appearance in higher plants. Figure 7 gives the formula for two representative members and table 17 lists the ones isolated so far in fractionation. Maytansine was isolated from several *Maytenus* species (17) and colubrinol from *Colubrina texensis* (Torr. & Gray) Gray (18). The group is generally cytotoxic and is unusually active in P388 leukemia at remarkably low doses. Maytansine, the most readily available member of the group, is also active in L1210 leukemia and B16 melanoma and is now in clinical trial. It is interesting to note that one of the *Maytenus* species, many of which are known to contain maytansine, has been in popular use for 30 years in South Africa in an herbal mixture for carcinoma and sarcoma.

Proteins

Over the years, proteinaceous materials have been isolated as the active agents in several plants (table 18). Some were thought to be simple proteins

ANSA MACROLIDES



Maytansine NSC-153858 R = R' = H

Colubrinol NSC-196519 R = CH_3; R' = OH

FIGURE 7

Table 16. - Quassinoids

	NSC	Active	Inactive
2'-Acetylglaucarubinone 11/	194251	PS (157), KB	
Ailanthinone 11/	238187	PS (148), KB	
Bruceantarin 11/	175399	PS (150), KB	
Bruceantin 11/	165563	B1, LE, PS (220), KB	LL(new)
Bruceantinol 11/	238177	LE, PS (145), KB	
Bruceine B 11/	132793	PS (130), KB	
Dehydroailanthinone 11/	238188	PS (184), KB	
Dehydrobruceantarin 11/	238179	PS (133), KB	
Dehydrobruceantin 11/	238178	PS (135)	KB
Dehydrobruceantol 11/	238180	KB	
Glaucarubinone 11,17/	132791	B1, PS (231), KB	LE, LL(new)
Glaucarubolone 11,17/	238189 126764	PS (150), KB	LE
Holacanthone 17/	126765	B1, PS (227), KB	LE, WA
Isobruceine B 11/	238181	PS (140), KB	LE

Table 17. - Ansa macrolides

	NSC	Active	Inactive
Colubrinol 17/	196519	PS (213), KB	
Colubrinol acetate 17/	196520	PS (206), KB	
Maysenine 11/	219974	KB	PS
Maysine 11/	219972	KB	PS
Maytanacine 11/	239387	PS (190), KB	
Maytanbutine 11,17/	165014	PS (190), KB	
Maytanprine 11/	165013	PS (161)	
Maytansine 11/	153858	B1, LE, PS (220), KB	LL(new)
Maytansinol 11/	239386	KB	
Maytanvaline 11/	219970	PS (201), KB	
Normaysine 11/	219973	KB	PS

Table 18. - Proteins

	NSC	Active	Inactive
Simple proteins (?)			
from Caesalpinia 2/	--	SA	
" Cerastium 2/	--	SA	
" Cercidium 2/	--	LL, WA	
" Gutierrezia 2/	--	SA	
" Mertensia 2/	--	SA	
Glycoproteins (?)			
Cesalin 2/	110435	LL, SA, WA, KB	B1, PS, LE, LL(new)
from Mirabilis 2/	--	LL, SA, WA	
" Muscari 15/	--	WA	
" Osteomeles 2/	--	SA	

and others were thought to be glycoproteins but, with one exception, they were never purified. The one with the highest activity in Walker 256, obtained from *Caesalpinia gilliesii* (Hook.) D. Dietr. in good yield (19), was selected for further development. Purification yielded a product (named cesalin), highly active in Walker 256 but inactive in the leukemias and slow-growing tumors, B16 melanoma and Lewis lung carcinoma (new). As a representative of a large class of natural products, it will be interesting to follow its progress in preclinical pharmacologic and possible clinical trials.

Alkaloids

More members of this class of compounds have come out of the plant fractionation program than of any other class. They are widely distributed in the plant kingdom and many are active against KB cells or Walker 256 carcinoma. After isolation, activity in other in vivo tumor systems has frequently been found. The great diversity of chemical types in this group of compounds is illustrated in table 19 which lists the classes into which the 80 isolated alkaloids (table 20) fall. No attempt will be made to draw any structure-activity relationships since the number of compounds is too small and the data are too scanty. Rather, a few of the alkaloids that have proved to be most interesting will be discussed. Figures 8–12 represent most of the interesting alkaloids in our program and typify the variety of the chemical structures encountered, some of which are novel.

Table 19. - Alkaloids

Class			
1.	Aliphatics	14.	Amaryllidaceae alkaloids
2.	Colchicine group	15.	Canthine group
3.	Pyrrolizidines	16.	Rauvolfia alkaloids
4.	Tetrahydroisoquinolines	17.	Isoquinuclidines
5.	Bisbenzylisoquinolines	18.	Dimeric indoles
6.	Aporphines	19.	Ellipticine group
7.	Dibenzopyrrocolines	20.	Taxanes
8.	Morphinane group	21.	Camptothecin group
9.	Protoberberines	22.	Furoquinolines
10.	Benzophenanthridines	23.	Sterol alkaloids
11.	Emetine group	24.	Cephalotaxus alkaloids
12.	Alangium alkaloids	25.	Anopterus alkaloids
13.	Phenanthroquinolizidines and phenanthroindolizidines	26.	Alkaloids of unknown structure

a. Thalicarpine (fig 8).—This is the first dimeric alkaloid recognized to contain both the aporphine and benzylisoquinoline moieties (20). It entered clinical trial primarily on the basis of its Walker 256 antitumor activity. Major organ toxicity is consistently manageable. While it is still under study in the clinic, however, its prospects do not look promising.

b. Indicine N-oxide (fig 8).—Many pyrrolizidine alkaloids have been tested besides the four reported here (6), but indicine *N*-oxide, from *Heliotropium indicum* L., is the only one possessing significant activity in P388 leukemia. Since it is also active against the B16 melanoma, L1210 leukemia, and Walker 256, it was entered into clinical trials. Although pharmacologic testing is as yet incomplete, negative histopathologic findings indicate that the hepatotoxicity commonly associated with this class of alkaloids may not be a clinical problem with this compound.

Heliotropium indicum has been used since ancient times against warts and its close relative, *H. europaeum* L., has been recommended for cancer treatment (1).

c. Camptothecin (fig 9).—This novel structure, isolated in minute quantity from the wood of *Camptotheca acuminata* Decne. (21), is highly active in P388 and L1210 leukemias but causes gastrointestinal tract toxicity in the mouse. Results from its first clinical trial in patients with gastrointestinal cancer looked very promising (22), but subsequent work did not uphold the earlier findings.

Camptothecin is present in the plants along with smaller quantities of the 10-hydroxy and 10-methoxy derivatives (23). Since these also have high activity in P388 and/or L1210 leukemias it is possible that one of them might yield better clinical results. Accordingly, a comparative advanced bioassay is being carried out on camptothecin, the two derivatives, and 9-methoxycamptothecin in order to provide a basis on which to select one for further development.

It is interesting that a plant not closely related, *Mappia foetida* (Wight) Miers, has yielded both camptothecin and 9-methoxycamptothecin (24); preliminary tests with the latter compound reveal activity similar to the 10-methoxy derivative.

d. Taxol (fig 9).—This novel compound was isolated from *Taxus brevifolia* Nutt. and other *T.* species (25). Because of its high activity in the tumors of interest, suitably large quantities are being prepared for further study.

e. Ellipticine (fig 10).—Ellipticine and its 9-methoxy derivative were isolated from *Excavatia coccinea* (Teysm. & Binnend.) Markgraf and *Ochrosia moorei* (F. Muell.) F. Muell. (26). Their high activity in the leukemias indicated the further development of one of them. Ellipticine was selected because of better activity by the oral route and better availability by large-scale synthesis. Pharmacologic studies of this compound are in progress, and clinical studies of the 9-methoxy derivative have been carried out in France (27).

f. Tylocrebrine (fig 10).—This alkaloid, obtained from *Tylophora crebriflora* S.T. Blake (28), has been through limited clinical trial. Irreversible and unmanageable central nervous system effects, not anticipated from preclinical work, precluded further clinical studies.

g. Nitidine and fagaronine (fig 10).—These closely related alkaloids, isolated from *Fagara macrophylla* (Oliv.) Engl. (29) and *F. zanthoxyloides* Lam. (30), respectively, showed activity in the Lewis lung

Table 20. Alkaloids

	Class b/	NSC	Active	Inactive
Anopterine a,3/	25	179172	KB	WA, LE
Berberine sulfate 1,4,11,17,20/	9	5355	B1, PS (131), KB	SA, LE, WA CA, LL, LL(new)
Camptothecin 4/17/	21	94600	LE, PS (250), WA, KB	
Chelidimerine 6/	10	--	KB	
Cocsulinine 6/	5	--	KB	
Colchicine 11/	2	757	PS (191), WA, LE, KB	B1, CA, LL LL(new), SA
Compound from Alangium 13/	26	92071	SA, WA, PS (126)	CA, LE
" " " 17/	26	--	CA, KB	
" " Dichroa 17/	26	--	PS (165), KB	
Compound B from Tylophora crebriflora 13/	13	85707	CA, LE	SA, WA
" C " " 13/13		85708	CA, LE, SA, WA	
" D " " 13/13		85709	LE, PS (177)	
" E " " 13/13		92070	WA	CA, LE, SA
Compound D from Tylophora indica 13/	13	100056	HE	LE
" E " " 13/	13	100057	HE	LE
Conessine hydrochloride 6/	23	32989	WA, KB	CA, LE, SA
Coptisine chloride 6/	9	119754	KB	
Crinamine 3/	14	88421	KB	
Cryptopleurine 6/	13	19912	KB	CA, LL, SA, B1, LE, PS, W
Cryptowolline iodide 3/	7	86342	KB	
Cycleadrine 11/	5	--	KB	
Cyclesamorine 11/	5	--	KB	WA
Cycleapeltine 11/	5	--	KB	WA
Cycloprotobuxine 11/	23	102244	WA, PS (145)	
Demecolcine 11/	2	3096, 403147	PS (150), WA LE, KB	B1, CA, LL LL(new), SA
3-Desmethylcolchicine 11/	2	172946	B1, PS (236), LE	LL(new)
Desmethyltylophorinine 13/	13	94739	LE, PS (146)	
(-)-Dicentrine 11/	6	--	KB	PS
Ellipticine 3/	19	71795	WA, LE, B1 PS (208), KB	LL(new)
Emetine hydrochloride 17/	11	33669	PS (232), LE, KB	B1, CA, LL, SA, WA
Fagaronine 6/	10	157993	PS (270), LE	B1, KB
N-Formyldesacetylcolchicine 11/	2	403142	LE, PS (190), WA, KB	B1, CA, LL(new) SA

Table 20. - Alkaloids (continued)

	Class	NSC	Active	Inactive
Harringtonine 14,17/	24	124147	LE, PS (304,	B1, LL(new)
			WA, KB	
Homoharringtonine 14/	24	141633	LE, PS (300),	B1, LL(new)
			KB	
10-Hydroxycamptothecin 17/	21	107124	WA, LE	
			PS (268)	
Indicine N-Oxide 13/	3	132319	WA, PS (262),	LL, LL(new),
			B1, LE	KB
Isochondodendrine 11/	5	77035	KB	WA, PS
Isoharringtonine 14/	24	141634	LE, PS (209),	
			KB	
Leurosine sulfate 6/	18	90636	LE, KB	
base		196522		
Liriodenine 7/	6	93681	KB	PS, SA
		215254		
Lunasine chloride 3/	22	80204	KB	LE, PS, WA,
				B1, LL(new)
Lycorine 3,17/	14	401360	KB	SA, CA, LE,
				LL, WA
9-Methoxycamptothecin 4/	21	176323	B1, LE,	
			PS (217),	
			LL(new)	
10-Methoxycamptothecin 17/	21	111533	LE	
5-Methoxycanthin-6-one 3/	15	88929	LL(new), KB	LE, PS, WA
Methoxydihydronitidine 17/	10	147789	PS (263), LE,	B1, LL(new)
			KB	
		146396		
9-Methoxyellipticine 3/	19	69187	PS (200), SA,	WA
			LE, KB	
Methoxyharringtonine 17/	24	--	PS (214)	
N-Methyldemecolcine 11/	2	403150	KB	
O-Methylfagaronine 6/	10	168201	PS (200), LE	B1, KB
4-Methylthiocanthin-6-one 3/	15	88928	KB	LE, WA
Monocrotaline 11/	3	28693	PS (133), WA,	LL, LE, KB
			B1, SA, CA	
Nitidine chloride 11,17/	10	146397	PS (260), LE,	B1, LL(new)
			KB	
Obamegin 1/	5	123123	KB	
Oxyacanthine 1,4/	5	93135	KB	LE, SA, WA
Oxynitidine 17/	10	135066	KB	PS

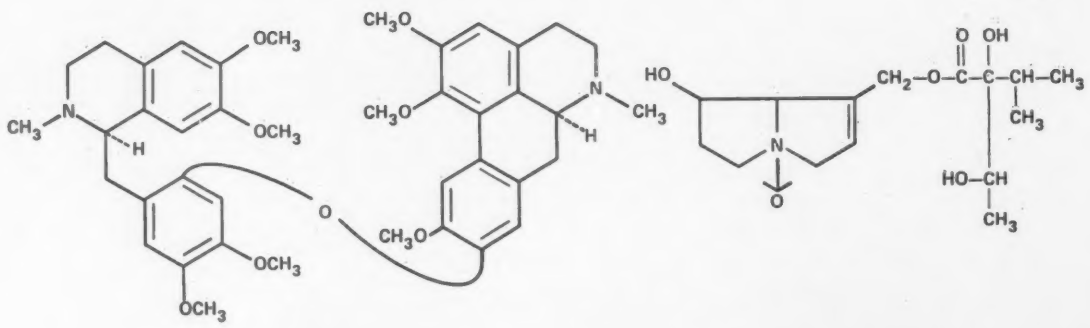
Table 20. - Alkaloids (continued)

	Class	NSC	Active	Inactive
Oxytylocrebrine 13/	13	85706	CA, LE, SA, PS (171), WA	
Pilocereine 17/	4	21075	KB	CA, LE, WA, SA, PS
Reserpine 3/	16	59272	SA, CA, WA	LE, B1, PS, KB
Sanguidimerine 6/	10	129231	KB	LE, PS, WA
Senecionine 11/	3	89935	WA, B1	LC, PS, KB
Senecionine N-oxide 11/	3	106677	WA	PS, LE, B1
β Solamarine 11/	23	94735	SA, WA	
Solapalmatenine 11/	1	123125	WA, KB	LE
Solapalmatine 11/	1	123124	WA, KB	LE
Solaplumbin 4/	23	--	WA	
Solasodine hydrochloride 4/	23	35543	WA, LL	CA, LE, PS, SA, KB
base		178260		
base		179187		
Solasodine rhamnoside 4/	23	--	WA	
epi-Stephanine 11/	5	121392	WA	PS, KB
Stephavanine 11/	8	135026	LL	
Taxol 17/	20	125973	WA, LE, B1 PS (190), KB	LL
a Tetrasol 17/	20	--	KB	PS
Thalicarpine 11/	6	68075	LL, PS (130), WA, KB	L1, CA, LE LL(new), SA
Thalidasine 11/	5	90285	WA	KB
Tubulosine 17/	12	131547	LE, PS (186), KB	B1
Tylocrebrine 3,10/	13	60387	CA, LE PS (170), KB	B1, LL, LL(new SA, WA
Tylophorine 18/	13	76387	LE, SA	CA, WA
Tylophorinine 13/	13	100055	LE	
Vosamine 10,13/	18	82591	WA, SA	CA, LE, LL PS, B1, KB
Vosorine 13/	18	92072	WA	LE, SA

a/ Superscripts in this column refer to supplier numbers in table 3.

b/ The classes are numbered according to table 19.

ALKALOIDS

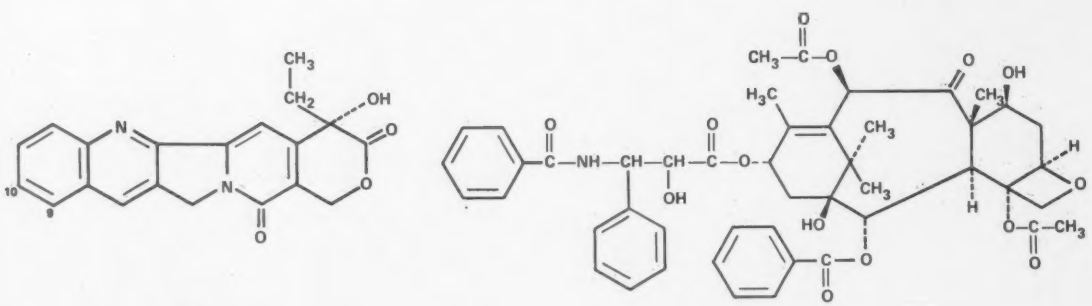


Thalicarpine
NSC-68075

Indicine N-oxide
NSC-132319

FIGURE 8

ALKALOIDS

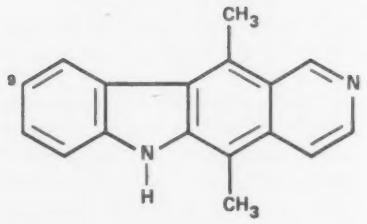


Camptothecin
NSC-94600

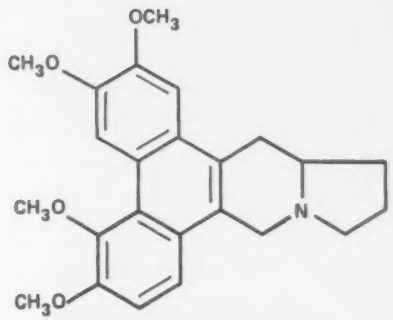
Taxol
NSC-125973

FIGURE 9

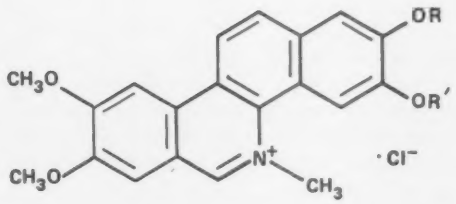
ALKALOIDS



Ellipticine
NSC-71795



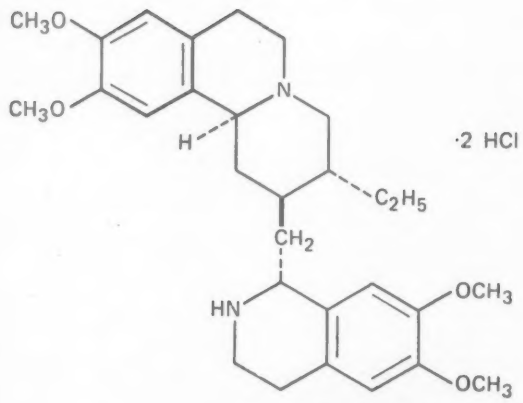
Tylocrebrine
NSC-60387



Nitidine chloride
NSC-146397 $RR' = CH_2$

Fagaronine
NSC-157995 $R = H; R' = CH_3$

FIGURE 10



Emetine hydrochloride

NSC-33669

FIGURE 11

(new) tumor. A simple addition reaction product of nitidine, methoxydihydronitidine, was obtained from the plant as an artifact (29) and showed similar activity. Advanced comparative screening is under way to determine which compound should be developed.

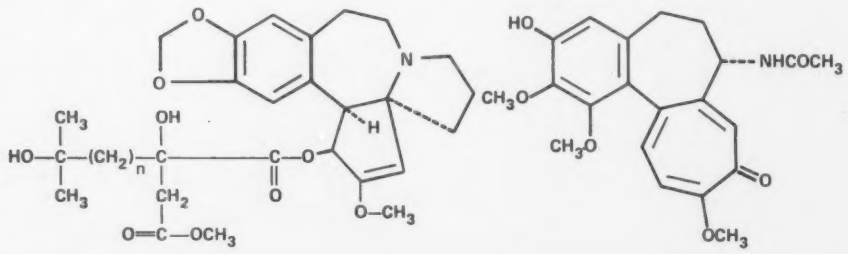
h. Emetine (fig 11).—This alkaloid, isolated in our program from *Cephaelis acuminata* Karst. (tables 3 and 20), but already an old commercial drug used as an amebicide, was given a clinical trial on the basis of its activity in P388 and L1210 leukemias. While there was some evidence of activity at high doses in lung carcinoma (≥ 50% reduction of tumor size in two cases) and in one case each of carcinoma of the trachea and of the thyroid gland (31), no response was demonstrated in several other malignancies (32).

i. Harringtonine and homoharringtonine (fig 12).—These alkaloids, together with isoharringtonine, isolated from *Cephalotaxus harringtonia* (Knight ex Forb.) R. Sm. var. *drupacea* (Sieb. & Zucc.) Koidz. (33), form a newly discovered type of active alkaloid which consists of complex esters of the inactive alcohol cephalotaxine. Lack of activity in the slow-growing tumors has lowered the priority on further development of these alkaloids, but the critical importance of the ester group for activity, a situation frequently encountered elsewhere, has stimulated efforts at synthesis designed to exploit the inherent activity of the esters; such efforts will hopefully provide compounds with broader activity.

j. 3-Desmethylcolchicine (fig 12).—This newly tested representative of an old class of active alkaloids was isolated from *Colchicum speciosum* Stev. (34). Certain advantages over other colchicine derivatives, one of which (demecolcine or V-deacetyl-V-methylcolchicine) has been declared (5) to be an established, clinically active antitumor agent, have revived interest in this group and sufficient quantities of this alkaloid are being obtained for further study.

Miscellaneous (table 21).—This table lists a small group of compounds that have antitumor activity but do not fall into one of the previous groups. None is particularly interesting except perhaps as an

ALKALOIDS



Harringtonine
NSC-124147
n = 2

Homoharringtonine
NSC-141633
n = 3

3-Desmethylcolchicine
NSC-172946

FIGURE 12

Table 21. - Miscellaneous

	NSC	Active	Inactive
Anacardic acid (?) 13/	106051	WA	
Anacardol (?) 13/	106050	WA	LE, LL, SA
Anemonin 17/	94101	KB	SA, LL, LE
			WA
Aristolochic acid 11/	11926	CA, SA	PS, LE, LL
			KB
	50413		
Bhilawanol (?) 13/	90250	WA	
Crotepoxide 11/	106396	LL, WA	PS, LE
5,7-Dihydroxy-8-methoxy-	251675	KB	
2-methylchromone 2/			
Dulcitol 6/	1944	PS (136)	CA, LE, SA
Gallic acid 12/	20103	KB	CA, LL, LE
			WA, PS, SA
Geiparvarin 19/	142227	PS (130), KB	B1, LE, LL
Gossypol 2/	56817	LL(new), WA	B1, CA, LE,
		PS (150)	SA, KB
Ipolearoside 4/	---	WA	
Jatropham 2/	177850	PS (125)	
Lignin 13/	115726	WA	LE, PS
Montanic acid monoglyceride 6/	---	PS (125)	KB
cis-1,8-Pentadecadiene 7/	138426	PS (127), WA	
1-Pentadecene 7/	77125	WA	LE, PS, SA
Rotenone 5,13,24/	3507	PS (155), KB	CA, WA, LE
			LL, SA
	26258		
Scopoletin 6/	403647	PS (133)	CA, LE, SA
			KB
T-Thujaplicin 2/	18805	KB	PS, WA, SA
			CA, LE, LL
	43333		
	402796		
1-Usnic acid 11/	5889	PS (135)	WA, B1, LE,
			LL, LL(new),
			KB
Uvaretin 2/	241906	PS (133), KB	B1

indicator of related compounds to acquire and test in the hope of finding improved activity. For readier reference, an alphabetical list (table 22) of all the compounds mentioned in the tables has been prepared.

COMMENTS AND CONCLUSIONS

The compounds which have been isolated from plants in the NCI program with activity against experimental tumors have been listed according to chemical type, along with the tumors against which they have shown activity and inactivity. Changes in methods of extraction and in the composition of the antitumor screen which have affected the identity and type of the compounds isolated are discussed.

It is evident that antitumor activity is encountered in compounds encompassing a wide variety of chemical classes. Many of these compounds are of novel chemical structure. On the basis of activity against experimental tumors thought to be most predictive of clinical effectiveness, it appears that certain chemical classes are of greater interest as a source of antitumor agents than others. Presently, the most interesting ones are the diterpenes, lignans, quassinoids, ansa macrolides, and alkaloids. Continued study of the plant kingdom will undoubtedly reveal other compounds in these classes that will be superior to existing ones, perhaps in possessing fewer toxic effects, and may also uncover other classes of compounds that will prove to be of high interest. In addition, it is expected that better plant sources of certain useful compounds will be found, as has been the case with maytansine.

A critical review of the antitumor properties of certain interesting compounds, as well as of some of the less interesting ones, will reveal fruitful areas for analog synthesis either from "scratch" or using existing compounds as templates. For example, a number of completely inactive alcohols have been converted into highly active esters simply by acylation.

Continued feedback of results from preclinical pharmacologic studies and from the clinic should result in modifications in the makeup of our bioassay screen and in the types of compounds that we are (and are not) seeking to test. Continued taxonomic analysis of plant sources of compounds should result in more selective collections for these compounds.

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C1	Acer saponin P	100045	14	P3
C2	Acer saponin Q	123429	14	P3
C3	2'-Acetylglaucarubinone	194251	16	P174, P175
C4	Acobioside A	116788	15	P7
C5	Acolongifloroside K	152149	15	P6
		173717		
C6	Acospectoside A	113569	15	P7
C7	Acovenoside A	116787	15	P6, P7
C8	Acovenoside B	116789	15	P7
C9	Adynerin	251673	15	P163
C10	Ailanthinone	238187	16	P175
C11	Alkannin	94524	6	P28
C12	Alkannin β,β-dimethylacrylate	140377	6	P28
C13	Alkannin monoacetate	140376	6	P28
C14	Allamandicin	251691	7	P15
C15	Allamandin	251690	7	P15
--	Aloe-emodin	See Emodin	6	
C16	Ambrosin	85235	8	P127
C17	α-Amyrin	114787	11	P49, P184
C18	Anacardic acid (?)	106051	21	P19
C19	Anacardol (?)	106050	21	P19
C20	Anemonin	94101	21	P20, P21, P22
C21	Anopterine	179172	20	P24
C22	Apocannoside	83216	15	P25
C23	Arctiopicrine	177853	8	P63
C24	Aristolochic acid	11926	21	P27
		50413		
C25	Artemisiifolin	177852	8	P63
C26	Baileyin	179192	8	P36
C27	Berberine sulfate	5355	20	P26, P40, P76, P125, P210, P231
C28	Bersaldegenin 3-acetate	135076	15	P41
C29	Bersaldegenin 1,3,5-orthoacetate	135077	15	P41
C30	Bersamagenin 1,3,5-orthoacetate	135032	15	P41
C31	Berscillogenin	--	15	P41
C32	Bersenogenin	251692	15	P41
C33	Betulin	4644	11	P17, P18
C34	Betulinic acid	113090	11	P128, P189, P224
C35	Bhilawanol (?)	90250	21	P190a
C36	Brucamtarin	175399	16	P44, P45
C37	Bruceantin	165563	16	P44, P45
C38	Bruceantinol	238177	16	P44, P45
C39	Brucein B	132793	16	P44, P45
--	Bufotalidin acetate	See Hellebrigenin 3-acetate	15	
C40	Burseran	123428	12	P47

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C41	Calotropin	143925 106393	15	P32
C42	Camptothecin	94600	20	P58, P152
C43	Celsioside C	173116	14	P63
C44	Centaureidin	106969	13	P35
C45	Cerberin	251674	15	P213
C46	Cesalin	110435	18	P53
C47	Chelidimerine	--	20	P69
C48	Cocculinine	251696	20	P73
C49	Colchicine	757	20	P74
C50	Colubrinol	196519	17	P75
C51	Colubrinol acetate	196520	17	P75
C52	Compound from Acanthospermum	--	8	P2
C53	" " Acer	--	14	P4
C54	" " Agave	--	14	P13
C55	" " "	137440	14	P13
C56	" " "	--	14	P13
C57	" " "	--	14	P13
C58	" " "	--	14	P11
C59	Compound from Agave	--	14	P12
C60	" " "	--	14	P10
C61	" " Alangium	92071	20	P14
C62	" " "	--	20	P14
C63	" " Allium	--	14	P16
C64	" " Asclepias	--	15	P31
C65	" " Aster	--	14	P33
C66	" " "	--	14	P34
C67	" " Bursera	--	11	P50
C68	" " Caesalpinia	--	18	P52
C69	" " Centaurea	--	8	P63
C70	" " Cerastium	--	18	P67
C71	" " Cercidium	--	18	P68
C72	" " Chrysopsis	--	14	P70
C73	" " Crossosoma	--	15	P80
C74	" " Cyclamen	135029	14	P86
C75	Compound from Dichroa	--	20	P91
C76	" " Elaeodendron	--	15	P96
C77	" " Entada	115727	14	P99
C77a	" " Gutierrezia	--	18	P115
C78	" " Hesperaloe	--	14	P122
C79	" " Ipomopsis	--	14	P131
C80	" " Jatropha	--	9	P134
C81	" " "	--	11	P134
C82	" " Machaeranthera	--	14	P149
C83	" " Maytenus	--	11	P156
C84	" " Mertensia	--	18	P158

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C85	" " Mirabilis	--	18	P159
C86	" " Muscari	--	18	P161
C87	" " Osteomeles	--	18	P167
C88	" " Rubia	--	11	P187
C89	" " Saponaria	77472	14	P188
C90	Compound from Solanum	--	14	P193
C91	" " Trillium	--	14	P216
C92	Compound B from Tylophora	85707	20	P218, P220
C93	" C " "	85708	20	P218, P220
C94	" D " "	85709	20	P218, P220
C95	" E " "	92070	20	P218
C96	" D " Tylophora indica	100056	20	P221
C97	" E " " "	100057	20	P221
C98	Conessine hydrochloride	32989	20	P124
C99	Coptisine chloride	119754	20	P69
C100	Coroglaucigenin	144150	15	P138
C101	Costunolide	106404	8	P142, P201
C102	Crinamine	88421	20	P79
C103	Crotepoxide	106396	21	P82
C104	Cryptopleurine	19912	20	P42
C105	Cryptowolline iodide	86342	20	P83
C106	Cucurbitacin B	49451	10	P39, P85, P89, P146, P153
C107	" D	521776	10	P39, P78, P84
C108	" D dehydroepirhamnoside	See Datiscocide	10	
C109	" E	106399 521775	10	P71, P153
C110	" F	--	10	P89
C111	" I	521777	10	P172
C112	" L	112167	10	P95
C113	" P	135074	10	P43
C114	" Q	135075	10	P43
C115	" glycoside	--	10	P89
C116	" "	--	10	P89
C117	" "	--	10	P89
C118	Cucurbitacin glycoside	--	10	P89
C119	Cycleadrine	--	20	P87
C120	Cycleanorine	--	20	P87
C121	Cycleapeltine	--	20	P87
C122	Cycloprotobuxine	102244	20	P51
C123	Cymarin	7522	15	P25, P168
C124	Damsin	85249	8	P110
C125	Datiscacin (Cucurbitacin R)	144154	10	P89
C126	Datiscoside (Cucurbitacin D dehydroepirhamnoside)	144153	10	P89
C127	Daucosterol (β-sitosterol glucoside)	165962	5	P177

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C128	Dehydroailanthinone	238188	16	P175
C129	Dehydroanhydropicropodophyllin	--	12	P46
C130	Dehydrobruceantarin	238178	16	P44
C131	Dehydrobruceantin	238178	16	P44, P45
C132	Dehydrobruceantol	238180	16	P44
C133	Demecolcine	3096 403147	20	P74
C134	3'-Demethylpodophyllotoxin	251681	12	P141
C135	Deoxypodophyllotoxin	403148	12	P46, P47, P48, P55, P121, P136, P141, P181, P214
C136	Desglucouzarin	--	15	P31
C137	5'-Desmethoxy-β-peltatin A methyl ether	126727	12	P46
C138	3-Desmethylcolchicine	172946	20	P74
C139	Desmethyltylophorinine	94739	20	P219, P221
C140	(-)-Dicentrine	--	20	P151
C141	Digitoxin	7529	15	P92
C142	Dihydrocucurbitacin B	106401	10	P39, P153
C143	5,7-Dihydroxy-8-methoxy-2-methylchromone	251675	21	P80
C144	(+)-Dimethylisolariciresinol-2α-xyloside	--	12	P41
C145	Dulcitol	1944	21	P156
C146	Elephantin	102817	8	P97
C147	Elephantopin	100046	8	P97
C148	Ellipticine	71795	20	P103, P165
C149	Emetine hydrochloride	33669	20	P64
C150	Emodin (aloe-emodin)	38628	6	P39, P185
C151	3-Epibersccillogenin	135067	15	P41
C152	2-Epicucurbitacin B	See Isocucurbitacin B	10	
C153	10-Epieupatoroxin	135068	8	P102
C154	Epitulipinolide	142844	8	P142
C155	Erioflorin	144151	8	P100
C156	Erioflorin acetate	251667	8	P179
C157	Erioflorin methacrylate	251666	8	P179
C158	Eriolangin	182855	8	P100
C159	Eriolanin	144152	8	P100
C160	Eupachlorin	114567	8	P102
C161	Eupachlorin acetate	114568	8	P102
C162	Eupachloroxin	114570	8	P102
C163	Eupacunin	135020	8	P101
C164	Eupacunoxin	135021	8	P101
C165	Euparotin	104942	8	P102
C166	Euparotin acetate	104943	8	P102
C167	Eupatocunin	135025	8	P101

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C168	Eupatofolin	135023	8	P101
C169	Eupatoroxin	114569	8	P102
C170	Eupatundin	114566	8	P102
C171	Fagaronine	157995	20	P109
C172	Fastigilin B	176503	8	P36
C173	Fastigilin C	176507	8	P36
C174	N-Formyldesacetylcolchicine	403142	20	P74
C175	Gaillardin	106394	8	P111
C176	Gallic acid	20103	21	P166, P186
C177	Geiparvarin	142227	21	P111a
C178	Gitoxigenin	407807	15	P83
C179	Glaucarubinone	132791	16	P174, P175, P191
C180	Glaucarubolone	238189 126764	16	P175, P191
C181	Gnidicin	238941	9	P112
C182	Gnididin	238942	9	P112
C183	Gniditrin	238943	9	P112
C184	Gossypol	56817	21	P114, P160
C185	Harringtonine	124147	20	P65, P66
C186	Helenalin	85236	8	P38, P117, P118
C187	Helenalin acetate	166124	8	P29
C188	Hellebrigenin 3-acetate (Bufotalidin acetate)	106676	15	P41
C189	Hellebrigenin 3,5-diacetate	109330	15	P41
C190	Holacanthone	126765	16	P123
C191	Homoharringtonine	141633	20	P65
C192	16β-Hydroxybersaldegenin 1-acetate	135080	15	P41
C193	16β-Hydroxybersaldegenin 3-acetate	135079	15	P41
C194	16β-Hydroxybersaldegenin 1,3,5-orthoacetate	135078	15	P41
C195	16β-Hydroxybersamagenin 1,3,5-orthoacetate	251695	15	P41
C196	10-Hydroxycamptothecin	107124	20	P58
C197	12-Hydroxydaphnetoxin	239073	9	P88
C198	Hyrcanoside	--	15	P77
C199	Indicine N-Oxide	132319	20	P120
C200	Ipolearoside	--	21	P129
C201	Isobruceine B	238181	16	P44
C202	Isochondodendrine	77035	20	P87
C203	Isocucurbitacin B (2-Epicucurbitacin B)	106400	10	P153
C204	Isogaillardin	106395	8	P111
C205	Isoharringtonine	141634	20	P65
C206	Isoplumericine	112153	7	P15
C207	Jacaranone	251682	6	P132
C208	Jatropham	177850	21	P134

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C209	Jatrophone	135037	9	P132
C210	Justicidin B	254665	12	P172a
C211	Lapachol	11905	6	P198
C212	Leurosine base	196522	20	P60, P51
	Leurosine sulfate	90636	20	
C213	Liatrin	135034	8	P139
C214	Lignin	115726	21	P98
C215	Lipiferolide	251676	8	P142
C216	Liriodenine	93681	20	P23
		215254		
C217	Lunasine chloride	80204	20	P147, P148
C218	Lupeol	90487	11	P17, P18, P50, P208
C219	Lycorine	401360	20	P79, P126
C220	Maysenine	219974	17	P154
C221	Maysine	219972	17	P154
C222	Maytanacine	239387	17	P154, P183
C223	Maytanbutine	165014	17	P75, P116, P154, P155, P157, P183
C224	Maytanprine	165013	17	P116, P154, P155, P157, P182, P183
C225	Maytansine	153858	17	P116, P154, P155, P157, P182, P183
C226	Maytansinol	239386	17	P154
C227	Maytanvaline	219970	17	P116, P154
C228	9-Methoxycamptothecin	176323	20	P152
C229	10-Methoxycamptothecin	111533	20	P58
C230	5-Methoxycanthin-6-one	88929	20	P171
C231	Methoxydihydronitidine	146396	20	P105, P106
		147789		
C232	9-Methoxyellipticine	69187	20	P103, P165
C233	Methoxyharringtonine	--	20	P66
C234	N-Methyldemecolcine	403150	20	P74
C235	O-Methylfagaronine	168201	20	P109
C236	4-Methylthiocanthin-6-one	88928	20	P171
C237	Mezerein	239072	9	P88
C238	Monocrotaline	28693	20	P81
C239	Montanic acid α-monoglyceride	--	21	P111b
C240	Myrsine saponin	123126	14	P162, P228
C241	Nardol diastereomer (?)	127084	8	P162a
C242	Neriifolin	123976	15	P211, P212, P213
C243	Nitidine chloride	146397	20	P104, P105, P106, P107, P108, P215, P231
C244	Normaysine	219973	17	P154
C245	Obamegin	123123	20	P229
C246	Oleandrigenin	148790	15	P83

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C247	Oleandrin	95089	15	P163
C248	Opposide	173716	15	P6
C249	Ovatifolin acetate	--	8	P179
C250	Oxyacanthine	93135	20	P40, P229
C251	Oxynitidine	135066	20	P106
C252	Oxytylocrebrine	85706	20	P218, P220
C253	Parthenolide	157035	8	P150
C254	Paucin	136722	8	P37
C255	α-Peltatin	24817	12	P30, P93
		35463		
C256	β-Peltatin	24819	12	P93
		35471		
C257	β-Peltatin A methyl ether	126726	12	P46
C258	cis-1,8-Pentadecadiene	138426	21	P95a
C259	1-Pentadecene	77125	21	P95a
C260	Pilocereine	21075	20	P144
C261	Plumericine	112152	7	P15
C262	Podolide	238978	9	P180
C263	Podophyllotoxin	24818	12	P135, P137, P141, P181
C264	Podophyllotoxin glucoside	163024	12	P56
C265	16-Propionylgitoxigenin	160843	15	P83
C266	Reserpine	59272	20	P103
C267	Rhodexin B	160845	15	P83
C268	Rotenone	8505	21	P90, P143, P176
		26258		
C269	Sanguidimerine	129231	20	P69
C270	Scilliglaucosidin	135036	15	P41, P222
C271	Scopoletin	405647	21	P119
C272	Senecionine	89935	20	P190
C273	Senecionine N-oxide	106677	20	P190
C274	β-Sitosterol	8096	5	P47, P57, P128, P130
		18173		P141, P145, P169
		49083		P170, P178, P184,
		86199		P208, P230
C275	β-Sitosterol glucoside	See Daucosterol	5	
C276	β-Solamarine	94735	20	P192
C277	Solapalmatenine	123125	20	P194
C278	Solapalmatine	123124	20	P194
C279	Solaplumbin	--	20	P164
C280	Solasodine base	178260	20	P164
		179187		
	Solasodine hydrochloride	35543	20	
C281	" rhamnoside	--	20	P164
C282	Somalin	251698	15	P8
C283	Steganacin	172958	12	P195
C284	Steganangin	172956	12	P195

Table 22. - Cumulative index of compounds listed in these tables and their plants of origin (see table 23) (continued)

Compound entry no.	Name	NSC No.	Table No.	Plant entry no. (table 23)
C285	Steganol	172959	12	P195
C286	Steganone	172957	12	P195
C288	epi-Staphanine	121392	20	P197
C289	Stephavanine	135026	20	P196
C290	Strophanthidin	86078	15	P168
C291	Taxifolin	36398	13	P94
C292	Taxodione	122419	6, 9	P202
C293	Taxodone	122420	6, 9	P202
C294	Taxol	125973	20	P203, P204, P205, P206, P207
C295	a Tetraol	--	20	P204
C296	Thalicarpine	68075	20	P209
C297	Thalidasine	90285	20	P209
C298	γ-Thujaplicin	18805	21	P1
		43338		
		402794		
C299	3',5,7-Trihydroxy-3,4'-dimethoxyflavone	106970	13	P35
C300	Tripdiolide	163063	9	P217
C301	Triptolide	163062	9	P217
C302	Triptonide	165677	9	P217
C303	Tubulosine	131547	20	P14
C304	Tulipinolide	106405	8	P142
C305	Tylocrebrine	60387	20	P218, P220
C306	Tylophorine	76387	20	P218, P220, P221
C307	Tylophorinine	100055	20	P218, P221
C308	Ursolic acid	4060	11	P9, P61, P163, P213, P224
C309	l-Usnic acid	5889	21	P72
C310	Uvaol	159627	11	P224
C311	Uvaretin	241906	21	P223
C312	Uzarigenin	119993	15	P113, P138
C313	Vernodalin	124459	8	P225
C314	Vernolepin	106398	8	P226
C315	Vernolide	124460	8	P225
C316	Vernomenin	116070	8	P226
C317	Vernomygdin	135072	8	P225
C318	Voacamine	82591	20	P199, P200, P227
C319	Voacorine	92072	20	P227
C320	Withacnistin	135073	15	P5
C321	Withaferin A	101088	15	P5, P173
C322	Zaluzanin C	177851	8	P230

Table 23. - Index of plants and active agents isolated therefrom

Plant entry no.	Name	Family	Compound entry no. (table 22)
P1	Abies concolor (Gord. & Glend.) Lindl. ex Hildebr.	Pinaceae	C298
P2	Acanthospermum glabratum (DC.) Wild	Asteraceae	C52
P3	Acer negundo L.	Aceraceae	C1, C2
P4	A. pensylvanicum L.	"	C53
P5	Dunalia arborescens (L.) Sleumer [Acnistus arborescens (L.) Schlecht.]	Solanaceae	C320, C321
P6	Acokanthera longiflora Stapf	Apocynaceae	C5, C7, C248
P7	A. oblongifolia (Hochst.) Codd	"	C4, C6, C7, C8
P8	Adenium obesum (Forskal) Roem. & Schult.	"	C282
P9	Adinandra dumosa Jack	Theaceae	C308
P10	Agave brandegeei Trel.	Agavaceae	C60
P11	A. caeciliana Berger	"	C58
P12	A. lecheguilla Torr.	"	C59
P13	A. schottii Engelm.	"	C54, C55, C56, C57
P14	Alangium salviifolium (L.fil.) Wanger.	Alangiaceae	C61, C62, C303
P15	Allamanda cathartica L.	Apocynaceae	C14, C15, C206, C261
P16	Allium drummondii Regel	Liliaceae	C63
P17	Alnus firmifolia Fern.	Betulaceae	C33, C218
P18	A. rubra Bong [A. oregona Nutt.]	"	C33, C218
P19	Anacardium occidentale L.	Anacardiaceae	C18, C19
P20	Pulsatilla patens (L.) Mill. [Anemone patens L.]	Ranunculaceae	C20
P21	P. pratensis (L.) Mill. [Anemone pratensis L.]	"	C20
P22	P. vulgaris Mill. [Anemone pulsatilla L.]	"	C20
P23	Annona glabra L.	Annonaceae	C216
P24	Anopterus macleayanus F.Muell.	Saxifragaceae	C21
P25	Apocynum cannabinum L.	Apocynaceae	C22, C123
P26	Argemone mexicana L.	Papaveraceae	C27
P27	Aristolochia indica L.	Aristolochiaceae	C24
P28	Arnebia nobilis Rech.fil.	Boraginaceae	C11, C12, C13
P29	Arnica chamissonis Less. subsp. foliosa (Nutt.) Maguire var. incana (Gray) Hult.	Asteraceae	C187
P30	Asarum canadense L.	Aristolochiaceae	C255
P31	Asclepias albicans Wats.	Asclepiadaceae	C64, C136
P32	A. curassavica L.	"	C41
P33	Aster divaricatus L.	Asteraceae	C65
P34	A. glaucodes Blake	"	C66
P35	Baccharis sarothroides Gray	"	C44, C299
P36	Baileya multiradiata Harv. & Gray ex Torr.	"	C26, C172, C173
P37	B. pauciradiata Harv. & Gray ex Gray	"	C254
P38	Balduina angustifolia (Pursh) Robins.	"	C186
P39	Begonia X tuberhybrida Voss cv. "Alba"	Begoniaceae	C106, C107, C142
P40	Berberis asiatica Roxb. ex DC.	Berberidaceae	C27, C250
P41	Beresma abyssinica Fresen.	Melianthaceae	C28, C29, C30, C31, C32, C144, C151, C188, C189, C192, C193, C194, C195, C270

Table 23. - Index of plants and active agents isolated therefrom (continued)

Plant entry no.	Name	Family	Compound entry no. (table 22)
P42	Boehmeria cylindrica (L.) Sw.	Urticaceae	C104
P43	Brandegea bigelovii (Wats.) Cogn.	Cucurbitaceae	C113, C114
P44	Brucea antidysenterica J.F.Mill.	Simaroubaceae	C36, C37, C38, C39, C130, C131, C132, C201
P45	B. guineensis G.Don	"	C36, C37, C38, C39, C129, C131
P46	Bursera fagaroides (H.B.K.) Engl.	Burseraceae	C135, C137, C257
P47	B. microphylla Gray	"	C40, C135, C274
P48	B. morelensis Ramirez	"	C135
P49	B. schlechtendalii Engl.	"	C17
P50	B. simaruba (L.) Sarg.	"	C67, C218
P51	Buxus sempervirens L.	Buxaceae	C122
P53	Caesalpinia gilliesii (Hook.) D.Dietr.	Fabaceae	C46
P54	C. pulcherrima (L.) Sw.	"	C68
P55	Callitris columellaris F.Muell.	Cupressaceae	C135
P56	Callitris drummondii (Parl.) F.Muell.	Cupressaceae	C264
P57	Calycogonium squamulosum Cogn.	Melastomataceae	C274
P58	Camptotheca acuminata Decne.	Nyssaceae	C42, C196, C229
P59	Cassia obtusa Clos	Fabaceae	C150
P60	Catharanthus lanceus (Boj. ex A.DC.) Pichon	Apocynaceae	C212
P61	C. pusillus (Murr.) G.Don	"	C212, C308
P62	Verbascum chinense (L.) Santapau [Celsia coromandeliana Vahl]	Scrophulariaceae	C43
P63	Centaurea melitensis L.	Asteraceae	C23, C25, C69
P64	Cephaelis acuminata Karst.	Rubiaceae	C149
P65	Cephalotaxus harringtonia (Knight ex Forb.) K.Sm. var. drupacea (Sieb. & Zucc.) Koidz.	Cephalotaxaceae	C185, C191, C205
P66	C. harringtonia cv. "Fastigiata"	"	C185, C233
P67	Cerastium texanum Britt.	Caryophyllaceae	C70
P68	Cercidium microphyllum (Torr.) Rose & Jtn.	Fabaceae	C71
P69	Chelidonium majus L.	Papaveraceae	C47, C99, C269
P70	Chrysopsis villosa (Pursh) Nutt. ex DC.	Asteraceae	C72
P71	Citrullus colocynthis (L.) Schrad.	Cucurbitaceae	C109
P72	Cladonia leptoclada desAbb.	Cladoniaceae	C309
P73	Cocculus pendulus (Forst.) Diels	Menispermaceae	C48
P74	Colchicum speciosum Stev.	Liliaceae	C49, C133, C138, C174, C234
P75	Colubrina texensis (Torr. & Gray) Gray	Rhamnaceae	C50, C51, C223
P76	Coptis teeta Wall.	Ranunculaceae	C27
P77	Coronilla varia L.	Fabaceae	C198
P78	Crinodendron hookerianum C.Gay	Elaeocarpaceae	C107
P79	Crinum macrantherum Engl.	Amaryllidaceae	C103, C219
P80	Crossosoma parviflorum Robins. & Fern.	Crossosomataceae	C73, C143
P81	Crotalaria spectabilis Roth	Fabaceae	C238
P82	Croton macrostachyus Hochst. ex Delile	Euphorbiaceae	C103
P83	Cryptocarya laevigata Bl. var. bowiei (Hook.) Kosterm. [C. bowiei (Hook.) Druce]	Lauraceae	C105

Table 23. - Index of plants and active agents isolated therefrom (continued)

Plant entry no.	Name	Family	Compound entry no. (table 22)
P84	Cryptostegia grandiflora R.Br.	Asclepiadaceae	C107, C178, C246, C265, C267
P85	Cucurbita digitata Gray	Cucurbitaceae	C106
P86	Cyclamen persicum Mill.	Primulaceae	C74
P87	Cyclea peltata (Lam.) Hook.fil. & Thoms.	Menispermaceae	C119, C120, C121, C202
P88	Daphne mezereum L.	Thymelaeaceae	C197, C237
P89	Datisca glomerata (Presl) Baill.	Datiscaceae	C106, C110, C115, C116, C117, C118, C119, C125, C126
P90	Derris trifoliata Lour.	Fabaceae	C268
P91	Dichroa febrifuga Lour.	Saxifragaceae	C75
P92	Digitalis purpurea L.	Scrophulariaceae	C141
P93	Diphylleia cymosa Michx.	Berberidaceae	C255, C256
P94	Drimys winteri Forst. & Forst.fil. var. chilensis (DC.) Gray	Winteraceae	C291
P95	Ecballium elaterium (L.) A.Rich	Curcurbitaceae	C112
P95a	Echinacea angustifolia DC.	Asteraceae	C258, C259
P96	Elaeodendron xylocarpum (Vent.) DC.	Celastraceae	C76
P97	Elephantopus elatus Bertol.	Asteraceae	C146, C147
P98	E. scaber L.	"	C214
P99	Entada phaseoloides (L.) Merr.	Fabaceae	C77
P100	Eriophyllum lanatum (Pursh) Forb.	Asteraceae	C155, C158, C159
P101	Eupatorium cuneifolium Willd.	"	C162, C163, C167, C168
P102	E. rotundifolium L.	"	C153, C160, C161, C162, C165, C166, C169, C170
P103	Bleekeria coccinea (Teysm. & Binnend.) Koidz. [Excavatia coccinea (Teysm. & Binnend.) Markgraf	Apocynaceae	C148, C232, C266
P104	Fagara chalybea (Engl.) Engl.	Rutaceae	C243
P105	F. leprieurii (Guillem., Perr. & A.Rich.) Engl.	"	C231, C243
P106	F. macrophylla (Oliv.) Engl.	"	C231, C243, C251
P107	Fagara rubrescens (Planch.) Engl.	Rutaceae	C243
P108	F. usambarensis Engl.	"	C243
P109	F. zanthoxyloides Lam.	"	C171, C235
P110	Ambrosia ambrosioides (Cav.) Payne [Franseria ambrosioides Cav.]	Asteraceae	C124
P111	Gaillardia pulchella Foug.	"	C175, C204
P111a	Geijera salicifolia Schott	Rutaceae	C177
P111b	Gnidia kraussiana Meissn.	Thymelaeaceae	C239
P112	G. lamprantha Gilg	"	C181, C182, C183
P113	Gomphocarpus physocarpus E.Meyer	Asclepiadaceae	C312
P114	Gossypium hirsutum L.	Malvaceae	C184
P115	Gutierrezia sarothrae (Pursh) Britt. & Rusby	Asteraceae	C77a
P116	Maytenus wightiana Babu [Gymnosporia rothiana (Wight & Arn.) Laws.]	Celastraceae	C223, C224, C225 C227
P117	Helenium autumnale L.	Asteraceae	C186
P118	H. microcephalum DC.	"	C186

Table 23. - Index of plants and active agents isolated therefrom (continued)

Plant entry no.	Name	Family	Compound entry no. (table 22)
P119	Helietta parvifolia (Gray ex Hemsl.) Benth.	Rutaceae	C271
P120	Heliotropium indicum L.	Boraginaceae	C199
P121	Hernandia ovigera L.	Hernandiaceae	C135
P122	Hesperaloe parviflora (Torr.) Coult.	Agavaceae	C78
P123	Holacantha emoryi Gray	Simaroubaceae	C190
P124	Holarrhena antidysenterica (L.) Wall. ex A.DC.	Apocynaceae	C98
P125	Hydrastis canadensis L.	Ranunculaceae	C27
P126	Hymenocallis latifolia (Mill.) M.J.Roem.	Amaryllidaceae	C219
P127	Hymenoclea salsola Torr. & Gray ex Gray	Asteraceae	C16
P128	Hyptis emoryi Torr.	Lamiaceae	C34, C274
P129	Ipomoea acuminata (Vahl) Roem. & Schult. [I. learii Paxt.]	Convolvulaceae	C200
P130	I. purpurea (L.) Roth	"	C274
P131	Ipomopsis aggregata (Pursh) V.Grant	Polemoniaceae	C79
P132	Jacaranda caucana Pittier	Bignoniaceae	C207
P133	Jatropha gossypiifolia L.	Euphorbiaceae	C209
P134	Jatropha macrorhiza Benth.	Euphorbiaceae	C80, C81, C208
P135	Juniperus chinensis L.	Cupressaceae	C263
P136	J. communis L. var. depressa Pursh	"	C135
P137	J. virginiana L.	"	C263
P138	Kanahia laniflolia (Forskal) R.Br.	Asclepiadaceae	C100, C312
P139	Liatris chapmanii Torr. & Gray	Asteraceae	C213
P140	Calocedrus decurrens (Torr.) Florin [Libocedrus decurrens Torr.]	Cupressaceae	C135
P141	Linum album Kotschy ex Boiss.	Linaceae	C134, C263, C274
P142	Liriodendron tulipifera L.	Magnoliaceae	C101, C154, C215, C304
P143	Lonchocarpus urucu Killip & A.C.Sm.	Fabaceae	C268
P144	Lophocereus schottii (Engelm.) Britt. & Rose	Cactaceae	C260
P145	Luffa echinata Roxb.	Cucurbitaceae	C274
P146	L. graveolens Roxb.	"	C106
P147	Lunasia amara Blanco	Rutaceae	C217
P148	Lunasia quercifolia (Warb.) K.Schum. & Lauterb.	Rutaceae	C217
P149	Machaeranthera linearis Greene	Asteraceae	C82
P150	Magnolia grandiflora L.	Magnoliaceae	C253
P151	M. virginiana L.	"	C140
P152	Mappia foetida (Wight) Miers	Icacinaceae	C42, C228
P153	Marah oregamus (Torr. & Gray) T.J.Howell	Cucurbitaceae	C106, C109, C142, C203
P154	Maytenus buchananii (Loes.) R.Wilczek	Celastraceae	C220, C221, C222, C223, C224, C225, C226, C227
P155	M. heterophylla (Eckl. & Zeyh.) N.Robson	"	C223, C224, C225
P156	M. senegalensis (Lam.) Exell	"	C83, C145
P157	M. serrata (Hochst. ex A.Rich.) R.Wilczek	"	C223, C224, C225
P158	Mertensia franciscana Heller	Boraginaceae	C84
P159	Mirabilis multiflora (Torr.) Gray	Nyctaginaceae	C85
P160	Montezuma speciosissima Sesse & Moc.	Malvaceae	C184

Table 23. - Index of plants and active agents isolated therefrom (continued)

Plant entry no.	Name	Family	Compound entry no. (table 22)
P161	Muscari comosum Mill.	Liliaceae	C96
P162	Myrsine africana L.	Myrsinaceae	C240
P162a	Nardostachys jatamansi DC.	Valerianaceae	C241
P163	Nerium oleander L.	Apocynaceae	C9, C247, C308
P164	Nicotiana plumbaginifolia Viv.	Solanaceae	C279, C280, C281
P165	Ochrosia moorei (F.Muell.) F.Muell.	Apocynaceae	C148, C232
P166	Oenothera caespitosa Nutt.	Onagraceae	C176
P167	Osteomeles schwerinae Schneid.	Rosaceae	C87
P168	Parquetina nigrescens (Afzel.) Bullock	Asclepiadaceae	C123, C290
P169	Parthenocissus inserta (A.J.Kern.) Fritsch	Vitaceae	C274
P170	Penstemon deustus Dougl. ex Lindl.	Scrophulariaceae	C274
P171	Pentaceras australis (F.Muell.) Hook.fil ex Benth.	Rutaceae	C230, C236
P172	Phormium tenax Forst. & Forst.fil	Agavaceae	C111
P172a	Phyllanthus brasiliensis (Aubl.) Poir.	Euphorbiaceae	C210
P173	Physalis virginiana Mill. var. sonorae (Torr.) Waterfall [P. longifolia Nutt.]	Solanaceae	C321
P174	Picrasma excelsa (Sw.) Planch.	Simaroubaceae	C3, C179
P175	Pierreodendron kerstingii (Engl.) Little	"	C3, C10, C128, C179, C180
P176	Piscidia erythrina (L.) Sarg.	Fabaceae	C268
P177	Pleocarpnus revolutus D.Don	Asteraceae	C127
P178	Plumeria acutifolia Poir.	Apocynaceae	C274
P179	Podanthus ovatifolius Lag.	Asteraceae	C156, C157, C249
P180	Podocarpus gracilior Pilg.	Podocarpaceae	C262
P181	Podophyllum pleianthum Hance	Berberidaceae	C135, C263
P182	Putterlickia pyracantha (L.) Endl.	Celastraceae	C224, C225
P183	P. verrucosa (E.Meyer ex Sonder) Szyszy.	"	C222, C223, C224, C225
P184	Pyrus pashia Buch.-Ham. ex D.Don	Rosaceae	C17, C274
P185	Rhamnus frangula L.	Rhamnaceae	C150
P186	Rhus trilobata Nutt.	Anacardiaceae	C176
P187	Rubia tenuifolia Urv.	Rubiaceae	C89
P188	Saponaria officinalis L.	Caryophyllaceae	C39
P189	Sarracenia flava L.	Sarraceniaceae	C34
P190	Senecio triangularis Hook.	Asteraceae	C272, C273
P190a	Semecarpus anacardium L.fil.	Anacardiaceae	C35
P191	Simarouba glauca DC.	Simaroubaceae	C179, C180
P192	Solanum dulcamara L.	Solanaceae	C276
P193	S. marginatum L.fil.	"	C90
P194	S. tripartitum Dun.	"	C277, C278
P195	Steganotaenia araliacea Hochst.	Apiaceae	C283, C284, C285, C286
P196	Stephania abyssinica (Dillon & A.Rich.) Walp.	Menispermaceae	C289
P197	S. japonica (Thunb. ex Murr.) Miers var. discolor (Blume) Forman [S. hernandiifolia (Willd.) Walp.]	"	C288
P198	Stereospermum suaveolens (Roxb.) DC.	Bignoniaceae	C211

Table 23. - Index of plants and active agents isolated therefrom (continued)

Plant entry no.	Name	Family	Compound entry no. (table 22)
P199	Tabernaemontana johnstonii (Stapf) Pichon	Apocynaceae	C318
P200	T. ventricosa Hochst. ex A.DC. [T. usambarensis K.Schum ex Engl.]	"	C318
P201	Talauma ovata St.-Hil.	Magnoliaceae	C101
P202	Taxodium distichum (L.) M.Rich.	Taxodiaceae	C292, C293
P203	Taxus baccata L. cv. "Fastigiata"	Taxaceae	C294
P204	T. brevifolia Nutt.	"	C294, C295
P205	T. canadensis Marsh.	"	C294
P206	T. cuspidata Sieb. & Zucc.	"	C294
P207	T. X media Rehd.	"	C294
P208	Teclea verdoorniana Exell & Mendonca [T. grandifolia Engl.]	Rutaceae	C218, C274
P209	Thalictrum dasycarpum Fisch., Mey. & Ave-Lall.	Ranunculaceae	C296, C297
P210	T. flavum L. subsp. glaucum (Desf.) Batt. [T. rugosum Ait.]	"	C27
P211	Thevetia ahouai (L.) A.DC.	Apocynaceae	C242
P212	T. ovata (Cav.) A.DC.	"	C242
P213	T. peruviana (Pers.) K.Schum.	"	C45, C242, C308
P214	Thuja occidentalis L.	Cupressaceae	C125
P215	Toddalia asiatica (L.) Lam.	Rutaceae	C243
P216	Trillium erectum L.	Liliaceae	C91
P217	Tripterygium wilfordii Hook.fil.	Celastraceae	C300, C301, C302
P218	Tylophora crebriflora S.T.Blake	Asclepiadaceae	C92, C93, C94, C95, C252, C305, C306, C307
P219	T. dalzellii Hook.fil.	"	C139
P220	T. hirsuta (Wall.) Wight	"	C92, C93, C94, C252, C305, C306
P221	T. indica (Burm.fil.) Merr.	"	C96, C97, C139, C306, C307
P222	Urginea altissima (L.fil.) Baker	Liliaceae	C270
P223	Uvaria acuminata Oliv.	Annonaceae	C311
P224	Vauquelinia corymbosa Correa ex Humb. & Bonpl.	Rosaceae	C34, C308, C310
P225	Vernonia amygdalina Delile	Asteraceae	C313, C315, C317
P226	V. hymenolepis A.Rich.	"	C314, C316
P227	Voacanga africana Stapf ex G.Ell.	Apocynaceae	C318, C319
P228	Wallenia yunquensis (Urban) Mez	Myrsinaceae	C240
P229	Xanthorrhiza simplicissima Marsh.	Ranunculaceae	C245, C250
P230	Zaluzania parthenioides (DC.) Rzed. [Z. robinsonii Sharp]	Asteraceae	C274, C322
P231	Zanthoxylum monophyllum (Lam.) P.Wils.	Rutaceae	C27, C243

Table 24. - Plant families and genera from Table 23

ACERACEAE
- Acer

AGAVACEAE
- Agave
- Hesperaloe
- Phormium

ALANGIACEAE
- Alangium

AMARYLLIDACEAE
- Crinum
- Hymenocallis

ANACARDIACEAE
- Anacardium
- Rhus
- Semecarpus

ANNONACEAE
- Annona
- Uvaria

APIACEAE
- Steganotaenia

APOCYNACEAE
- Acokanthera
- Adenium
- Allamanda

APOCYNACEAE
- Apocynum
- Bleekeria
- Catharanthus
- Holarrhena
- Nerium
- Ochrosia
- Plumeria
- Tabernaemontana
- Thevetia
- Voacanga

ARISTOLOCHIACEAE
- Aristolochia
- Asarum

ASCLEPIADACEAE
- Asclepias
- Cryptostegia
- Gomphocarpus
- Kanahia
- Parquetina
- Tylophora

ASTERACEAE
- Acanthospermum
- Ambrosia
- Arnica
- Aster
- Baccharis
- Baileya
- Balduina
- Centaurea
- Chrysopsis
- Echinacea
- Elephantopus
- Eriophyllum
- Eupatorium
- Gaillardia
- Gutierrezia
- Helenium
- Hymenoclea
- Liatris
- Machaeranthera
- Pleocarphus
- Podanthus
- Senecio
- Vernonia
- Zaluzania

BEGONIACEAE
- Begonia

BERBERIDACEAE
- Berberis
- Diphylleia
- Podophyllum

BETULACEAE
- Alnus

BIGNONIACEAE
- Jacaranda
- Stereospermum

BORAGINACEAE
- Arnebia
- Heliotropium
- Mertensia

BURSERACEAE
- Bursera

BUXACEAE
- Buxus

CACTACEAE
- Lophocereus

CARYOPHYLLACEAE
- Cerastium
- Saponaria

CELASTRACEAE
- Elaeodendron
- Maytenus
- Putterlickia
- Tripterygium

CEPHALOTAXACEAE
- Cephalotaxus

CLADONIACEAE
- Cladonia

COMPOSITAE
- See Asteraceae

CONVOLVULACEAE
- Ipomoea

CROSSOSOMATACEAE
- Crossosoma

CUCURBITACEAE
- Brandegea
- Citrullus
- Cucurbita
- Ecballium
- Luffa
- Marah

CUPRESSACEAE
- Callitris
- Calocedrus
- Juniperus
- Thuja

DATISCACEAE
- Datisca

ELAEOCARPACEAE
- Crinodendron

EUPHORBIACEAE
- Croton
- Jatropha
- Phyllanthus

FABACEAE
- Caesalpinia
- Cassia
- Cercidium
- Coronilla
- Crotalaria
- Derris
- Entada
- Lonchocarpus
- Piscidia

HERNANDIACEAE
- Hernandia

Table 24. - Plant families and genera from Table 23 (continued)

ICACINACEAE
- Mappia

LABIATAE
- See Lamiaceae

LAMIACEAE
- Hyptis

LAURACEAE
- Cryptocarya

LEGUMINOSAE
- See Fabaceae

LILIACEAE
- Allium
- Colchicum
- Muscari
- Trillium
- Urginea

LINACEAE
- Linum

MAGNOLIACEAE
- Liriodendron
- Magnolia
- Talauma

MALVACEAE
- Gossypium
- Montezuma

MELASTOMATACEAE
- Calycogonium

MELIANTHACEAE
- Bersama

MENISPERMACEAE
- Cocculus
- Cylcea
- Stephania

MYRSINACEAE
- Myrsine
- Wallenia

NYCTAGINACEAE
- Mirabilis

NYSSACEAE
- Camptotheca

ONAGRACEAE
- Oenothera

PAPAVERACEAE
- Argemone
- Chelidonium

PINACEAE
- Abies

PODOCARPACEAE
- Podocarpus

POLEMONIACEAE
- Ipomopsis

PRIMULACEAE
- Cyclamen

RANUNCULACEAE
- Coptis
- Hydrastis
- Pulsatilla
- Thalictrum
- Xanthorrhiza

RHAMNACEAE
- Colubrina
- Rhamnus

ROSACEAE
- Osteomeles
- Pyrus
- Vauquelinia

RUBIACEAE
- Cephaelis
- Rubia

RUTACEAE
- Fagara
- Geijera
- Helietta
- Lunasia
- Pentaceras
- Teclea
- Toddalia
- Zanthoxylum

SARRACENIACEAE
- Sarracenia

SAXIFRAGACEAE
- Anopterus
- Dichroa

SCROPHULARIACEAE
- Digitalis
- Penstemon
- Verbascum

SIMAROUBACEAE
- Brucea
- Holacantha
- Picrasma
- Pierreodendron
- Simarouba

SOLANACEAE
- Dunalia
- Nicotiana
- Physalis
- Solanum

TAXACEAE
- Taxus

TAXODIACEAE
- Taxodium

THEACEAE
- Adinandra

THYMELAEACEAE
- Daphne
- Gnidia

UMBELLIFERAE
- See Apiaceae

URTICACEAE
- Boehmeria

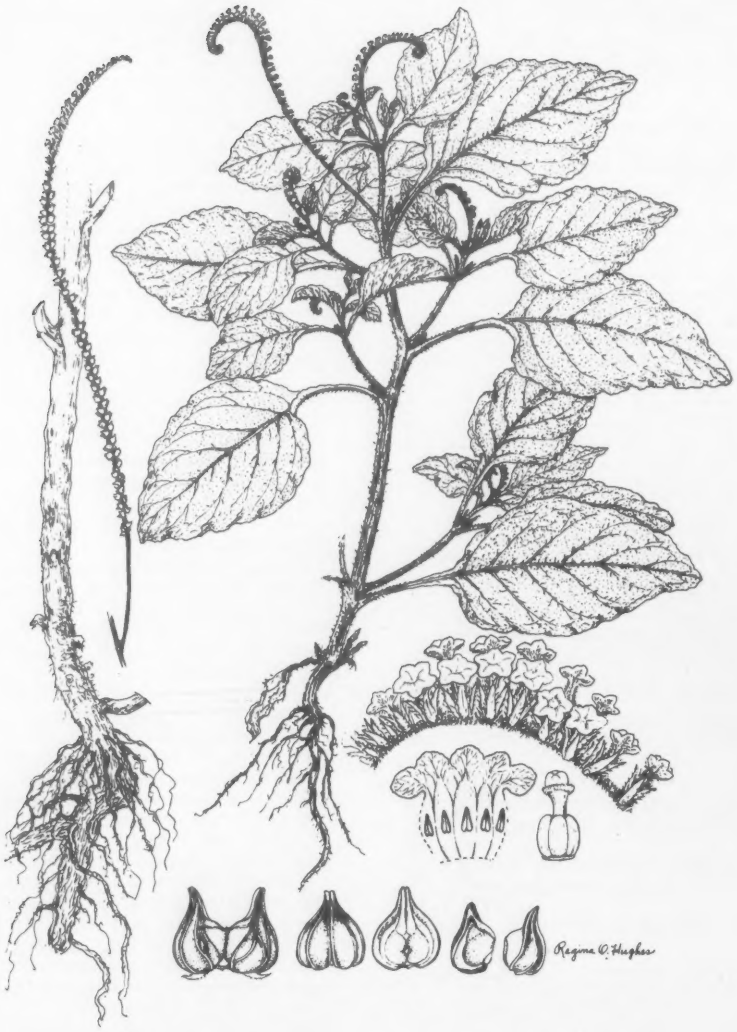
VALERIANACEAE
- Nardostachys

VITACEAE
- Parthenocissus

WINTERACEAE
- Drimys

REFERENCES

1. HARTWELL JL. Plants used against cancer. A survey. Lloydia 30:379–436, 1967, and ten additional installments ending with 34:386–437, 1971.
2. HARTWELL JL, and SCHRECKER AW. The chemistry of podophyllum. *In* Progress in the Chemistry of Organic Natural Products (Zechmeister L, ed). Vienna, Springer Verlag, 1958, vol 15, pp 83–166.
3. MAINE WRITERS RESEARCH CLUB. Maine Indians in History and Legends. Portland, Me, Severn-Wylie-Jewett Co, 1952, pp 96–102.
4. KOST J. The Elements of Materia Medica and Therapeutics. Cincinnati, Kost and Pool, 1849, 572 pp.
5. GOLDIN A, SERPICK AA, and MANTEL N. A commentary. Experimental screening procedures and clinical predictability value. Cancer Chemother Rep 50:173–218, 1966.
6. HARTWELL JL, and ABBOTT BJ. Antineoplastic principles in plants: recent developments in the field. *In* Advances in Pharmacology and Chemotherapy (Garattini S, Goldin A, Hawking F, et al, eds). New York, Academic Press, Inc, 1969, vol 7, pp 117–209 (*see* pp 154 and 194).
7. WALL ME, TAYLOR H, AMBROSIO L, ET AL. Plant antitumor agents. III. A convenient separation of tannins from other plant constituents. J Pharm Sci 58:839–841, 1969.
8. BLOCK JB, SERPICK AA, MILLER W, ET AL. Early clinical studies with lapachol (NSC-11905). Cancer Chemother Rep Part 2, vol 4(4):27–28, 1974.
9. KUPCHAN SM, DESSERTINE AL, BLAYLOCK BT, ET AL. Isolation and structural elucidation of allamandin, an antileukemic iridoid lactone from *Allamanda cathartica*. J Org Chem 39: 2477–2482, 1974.
10. KUPCHAN SM, AYNEHCHI Y, CASSADY JM, ET AL. The isolation and structural elucidation of two novel sesquiterpenoid tumor inhibitors from *Elephantopus elatus*. J Am Chem Soc 88:3674–3676, 1966.
11. KUPCHAN SM, COURT WA, DAILEY RG, JR, ET AL. Triptolide and tripdiolide. Novel antileukemic diterpenoid triepoxides from *Tripterygium wilfordii*. J Am Chem Soc 94:7194–7195, 1972.
12. DOSKOTCH RW, MALIK MY, and BEAL JL. Cucurbitacin B, the cytotoxic principle of *Begonia tuberhybrida* var. *alba*. Lloydia 32:115–122, 1969.
13. NISSEN NI, LARSEN V, PEDERSEN H, ET AL. Phase I clinical trial of a new antitumor agent, 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 56:769–777, 1972.
14. KUPCHAN SM, TAKASUGI M, SMITH RM, ET AL. Tumor inhibitors. LXII. The structures of acerotin and acerocin, novel tripterpene ester aglycones from the tumor inhibitory saponins of *Acer negundo*. J Org Chem 36:1972–1976, 1971.
15. KUPCHAN SM, BRITTON RW, ZIEGLER MF, ET AL. Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J Org Chem 38:178–179, 1973.
16. WALL ME, and WANI M. The isolation and structure of holacanthone, a potent experimental antitumor agent. (Abstr) *In* Proceedings of the 7th International Symposium on Chemical Natural Products, Riga, USSR, 1970, E138, p 72.
17. KUPCHAN SM, KOMODA Y, COURT WA, ET AL. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J Am Chem Soc 95:1354–1356, 1972.
18. WANI MC, TAYLOR HL, and WALL ME. Plant antitumor agents: colubrinol acetate and colubrinol, antileukaemic ansa macrolides from *Colubrina texensis*. J Chem Soc Chem Commun 470:390, 1973.
19. ULUBELEN A, MCCAUGHEY WF, and COLE JR. Proteinaceous antitumor substances from plants. III. *Caesalpinia gilliesii* (Leguminosae). J Pharm Sci 56:914–916, 1967.
20. KUPCHAN SM, and YOKOYAMA N. The structure, configuration and synthesis of thalicarpine, a novel dimeric aporphine-benzylisoquinoline alkaloid. J Am Chem Soc 85:1361–1362, 1963.
21. WALL ME, WANI MC, COOK CE, ET AL. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890, 1966.
22. GOTTLIEB JA, GUARINO AM, CALL JB, ET AL. Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). Cancer Chemother Rep 54:461–470, 1970.
23. WANI MC, and WALL ME. Plant antitumor agents. II. The structure of two new alkaloids from *Camptotheca acuminata*. J Org Chem 34:1364–1367, 1969.
24. GOVINDACHARI TR, and VISWANATHAN N. 9-Methoxycamptothecin. A new alkaloid from *Mappia foetida* Miers. Indian J Chem 10:453–454, 1972.
25. WANI MC, TAYLOR HL, WALL ME, ET AL. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93:2325–2327, 1971.
26. LODER JW. The structure of methoxyellipticine. Aust J Chem 19:1947–1950, 1966.
27. MATHE G, HAYAT M, DE VASSAL F, ET AL. Methoxy-9-ellipticine lactate. III. Clinical screening: its action in acute myeloblastic leukaemia. Rev Eur Etud Clin Biol 15:541–545, 1970.
28. GELLERT E, GOVINDACHARI TR, LAKSHMIKANTHAM MV, ET AL. The alkaloids of *Tylophora crebriflora;* structure and synthesis of tylocrebrine, a new phenanthroindolizidine alkaloid. J Chem Soc, pp 1008–1014, 1962.
29. WALL ME, WANI MC, and TAYLOR HL. Plant anti-tumor agents. VIII. Isolation and Structure of Anti-tumor Alkaloids from *Fagara macrophylla*. (Abstr) *In* Proceedings of the 162nd National Meeting of the American Chemical Society, Washington, DC, Sept 12–17, 1971. MEDI 34.
30. MESSMER WM, TIN-WA M, FONG HHS, ET AL. Fagaronine, a new tumor inhibitor isolated from *Fagara zanthoxyloides* Lam. (Rutaceae). J Pharm Sci 61:1858, 1972.
31. PANETTIERE F, and COLTMAN CA, JR. Phase I experience with emetine hydrochloride (NSC-33669) as an antitumor agent. Cancer 27:835–841, 1971.
32. SIDDIQUI S, FIRAT D, and OLSHIN S. Phase II study of emetine (NSC-33669) in the treatment of solid tumors. Cancer Chemother Rep 57:423–428, 1973.
33. POWELL RG, WEISLEDER D, SMITH CR, JR, ET AL. Structure of cephalotaxine and related alkaloids. Tetrahedron Lett 46:4081–4084, 1969.
34. KUPCHAN SM, BRITTEN RN, CHIANG CK, ET AL. Tumor inhibitors. 88. The antileukemic principles of *Colchicum speciosum*. Lloydia 36:338–340, 1973.



Magnification of Plant Parts:

Habit x ½
Young plant in early bloom stage
Left: part of old plant, showing roots and a fruiting spike

Flowers x 4
Flower opened to show sessile stamens x 8
Ovary x 10
Fruit, left to right x 5
Entire schizocarp—back and face views of schizocarp split in half
Two views of the single nutlet

Heliotropium indicum L.—The source of indicine-*n*-oxide, this is an annual herb and a common weed in many tropical and subtropical areas. It is easily grown from seed in the southeastern United States. The active agent is concentrated in the fruit, leaves, and young green stems.

Lower Plants as a Source of Anticancer Drugs

John D. Douros[1,*]

SUMMARY

The NCI's antineoplastic fermentation program is reviewed. Clinically useful antitumor antibiotics are discussed in terms of their activity, mechanism of action, and the producing microbe.

[Cancer Treat Rep 60:1069–1080, 1976]

Microbes have played an important part in man's history for centuries by causing disease, leaching minerals, fixing nitrogen, producing antibiotics for the treatment of disease, and producing commercially important chemicals. In 1864 Pasteur discovered that acetic acid could be produced by fermentation from ethanol. The ability of microbes to produce antimicrobial substances was first recognized by Fleming who observed the inhibition of staphylococcus on a plate contaminated with the fungus *Penicillium*. The therapeutic effects of penicillin were discovered by Florey et al in 1941. At that time the yield of penicillin was 2 units/ml; today, after years of technologic progress in fermentation, it is approximately 7000 units/ml.[2]

Actinomycin D, the first antitumor antibiotic, was isolated by Waksman and Woodruff in 1940. Japanese workers began an active search for antitumor antibiotics in 1951. Phleomycin, Umezawa's first major antitumor antibiotic, reached the clinic in 1956, but its evaluation was discontinued because of high renal toxicity.[3] In the same year, Hata discovered the mitomycins. In 1965, clinical studies were initiated in Japan with bleomycin which is presently used in the treatment of squamous cell carcinoma.

Umezawa postulated that antibiotics produced by microbes would inhibit the biosynthesis of nucleic acids, amino acids, proteins, or some essential cellular constituent of the cancer cell. He thought new antitumor antibiotics could be more easily identified with biochemical screens than with in vivo animal tests (1). Therefore, in 1965 Umezawa began using initial enzyme inhibition assays instead of in vivo antitumor testing for screening potential antineoplastic antibiotics (2).

The NCI fermentation program was initiated in 1956. In the early years of the antibiotic era a great deal of attention was given to fungi as a possible source of antibiotics (3). Undoubtedly the production of penicillin by *Penicillium chrysogenum* played a significant role in the decision to screen thousands of fungi between 1940 and 1950 (4).

Following the discovery of streptomycin, chloramphenicol, chlorotetracycline, and neomycin the emphasis has continued toward the streptomyces. The most successful method for obtaining new antibiotics has been the systematic examination of soil microorganisms, a technique yielding > 2300 antibiotics to date (5). Seventy percent of these antibiotics are produced by streptomyces. However, only 80 antibiotics are manufactured at present and only a handful are clinically effective therapeutic agents, eg, penicillin, cephalosporin, macrolides, lincomycin, tetracyclines, and polypeptides.

Fermentation in classic microbial physiology is defined as the anaerobic dissimilation of carbohydrates with the evolution of gas. Today, the term fermentation is used to describe almost any manufacturing operation using microorganisms. It includes anaerobic and aerobic processes, breakdown and build-up of carbon skeletons, and utilization of many types of molecules including carbohydrates with or without the evolution of gas.

In any fermentation process several biochemical reactions occur which constitute the metabolism of the microorganism involved. In essence, a microbe is virtually an enzyme factory which can grow on various substrates and produce different products.

[1]Drug Research and Development Program (DR&DP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

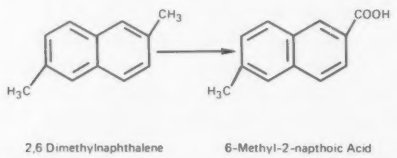
[2]Donovick R. Personal communication from Director, American Type Culture Collection, 1975.

[3]Umezawa H. Personal communication, Aug-Sept, 1974.

**Reprint requests to:* Dr. John D. Douros, DR&DP, DCT, NCI, Blair Bldg, Rm 4A15, 8300 Colesville Rd, Silver Spring, Md 20910.

There are two types of fermentation processes, conversion and biosynthesis. In the former type, a product is formed by the microbe which makes one or more chemical modifications of the substrate molecule. The carbon skeleton of the product is either identical with, or only slightly altered from, the carbon skeleton of the substrate. Figure 1 shows a typical microbial hydrocarbon conversion. The possibility of converting the antineoplastic antibiotic daunorubicin to adriamycin or modifying the aglycone by microbes seems quite logical (fig 2). Presently, adriamycin is produced by a mutant of the daunorubicin producer. Due to the apparent resistance of the A ring of the aglycone to chemical change, a microbial conversion seems to be a likely way to modify the A ring. Modifications of the aglycone might increase the activity or lower the cardiac toxicity of these highly promising anticancer antibiotics.

In fermentations of the conversion type, carbon compounds (excluding the substrate) such as glucose or hexadecane often serve as sources of energy and carbon for cellular metabolism and for synthesizing the enzymes which catalyze various reactions (eg, daunorubicin to adriamycin). Co-metabolism is the term used to identify a type of conversion fermentation utilizing one carbon source for growth and later adding a second carbon source whose structure will be modified. Yields are extremely high and far beyond that of a normal biosynthetic-type fermentation. Figure 3 indicates a typical co-metabolism reaction. It has been reported that yields of 20 g/liter of salicylic acid were obtained with this type of fermentation. Figure 4 shows some of the fermentation reactions microbes may carry out (6), many of which occur simultaneously. The initial step involves adding the chemical to be transformed at various times in the cell-population cycle. It is often essential to check for chemical modifications of the compound 4–12 hours later. Microorganisms are well suited for co-metabolism fermentation because of their many enzymes and specificity of the reactions. Thus, protection of other reactive groups on the molecule is not essential as it is with chemical manipulations.



2,6 Dimethylnaphthalene 6-Methyl-2-napthoic Acid

FIGURE 1.—Hydrocarbon fermentation conversion.

In biosynthetic fermentation the microbe carries out a complicated series of chemical reactions to yield a product with a carbon skeleton quite different from the starting raw material (fig 5). The product yields are usually much smaller in biosynthetic than in conversion fermentations. In the former type of fermentation, glucose is a source of carbon and energy for the growth of cells and is used to form the product of interest, in this case actinomycin D.

The NCI fermentation program is carried out through contracts. Because of their extensive experience in fermentation research, large multidisciplinary staffs with the latest equipment are sought. Industrial companies are most frequently involved in the search for antibiotics; pharmaceutic companies have identified and isolated > 2300 antibiotics, approximately 20% of which have been evaluated for antineoplastic activity.

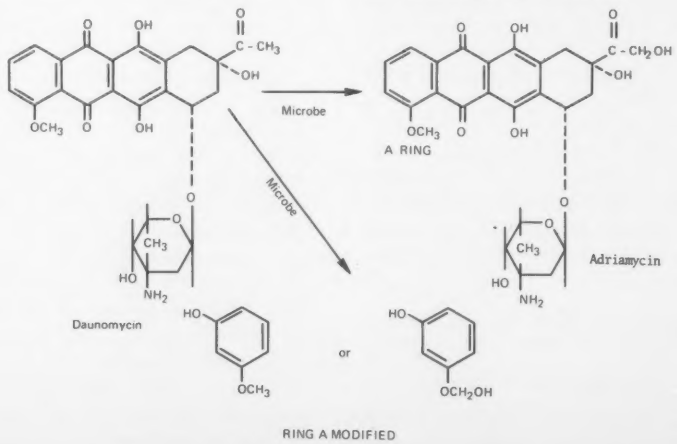


FIGURE 2.—Possible daunorubicin conversion.

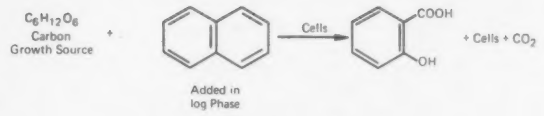


FIGURE 3.—Typical co-metabolism.

Figure 6 shows a flow chart of the NCI's fermentation program (7). The time frame and numbers of cultures are estimations. Since the inception of the program, approximately 160,000 primary culture fermentations have been evaluated. Normally 7–10 years are required for an antitumor antibiotic to be isolated, identified, scaled-up for production, and evaluated in preclinical toxicology, and for filing for and obtaining the investigational new drug (IND) application and clinical data. This closely approximates the time frame for commercialization of most antimicrobial antibiotics.

Before a novel antineoplastic antibiotic is discovered, many cultures must be evaluated and the discard rate is high. For example, in order for three antibiotics to reach the stage of IND application, ≤ 5000 new fermentations must be evaluated. Although heavy emphasis has been placed on streptomyces and conventional fermentation techniques for the past 15 years, marked changes are now being initiated in the fermentation program in the hope that they will increase the rate at which novel antineoplastic drugs are uncovered. Unique substrates, wide variations in environmental conditions, varying isolation techniques, and biotransformation and co-metabolism fermentation studies are now being evaluated as potential techniques for inducing cultures to produce new antibiotics.

Contractors have begun the evaluation of a number of unique microbes including thermophiles, psychrophiles, and osmophiles. Figure 7 indicates some of the substrates and parameters which will be studied in order to obtain novel organisms and new antibiotics.

The antibiotic industry has traditionally used streptomyces growing on a sugar medium. Microbiologic studies in the 1960s showed that various growing organisms could utilize as carbon sources

1. Oxidation	10. Dismutation
2. Reduction	11. Condensation
3. Decarboxylation	12. Amination
4. Deamination	13. Acylation
5. Glycosylation	14. Amidation
6. Hydrolysis	15. Phosphorylation
7. Alkylation	16. Transamination
8. Esterification	17. Isomerization
9. Dehydration	18. Halogenation

FIGURE 4.—Fermentation-type reactions.

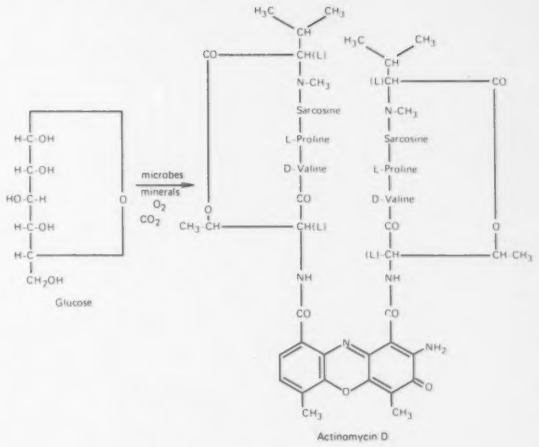


FIGURE 5.—Biosynthesis fermentation.

the following substances: hydrocarbons, terpenes, rubber, and complex chemicals. These fermentations yielded a variety of products, such as amino acids, cinnamic acid, salicylic acid, beta carotene, single cell protein, etc.

Streptomyces have been an excellent source of substances with antibiotic activity against some types of bacteria. Nevertheless, under test conditions used in the past, the streptomyces have not been a good source of antifungal or antiviral substances.[4]

Taxonomic key (8)

Order: Actinomycetales

Family 1:	Actinomycetaceae	Family 4:	Actinoplanaceae
Genera:	Actinomyces	Genera:	Actinoplanes
	Arachnia		Spirillospora
	Bifidobacterium		Streptosporangium
Family 6:	Nocardiaceae		Amorphosporangium
Genera:	Nocardia	Family 7:	Streptomycetaceae
	Pseudonocardia	Genera:	Streptomyces
			Streptoverticillium
			Sporichthya
			Microellobosporia

The question thus arises as to the wisdom of placing such emphasis on the streptomycetes in the search for antitumor agents from microbes. Although the NCI's program is not designed to obtain primarily streptomyces, these organisms play a major role in the program. When antibiotics from the actinomycetales are referred to, it should be kept in mind that the vast majority of organisms tested were indeed streptomyces but relatively few of the

[4]*See* footnote 2.

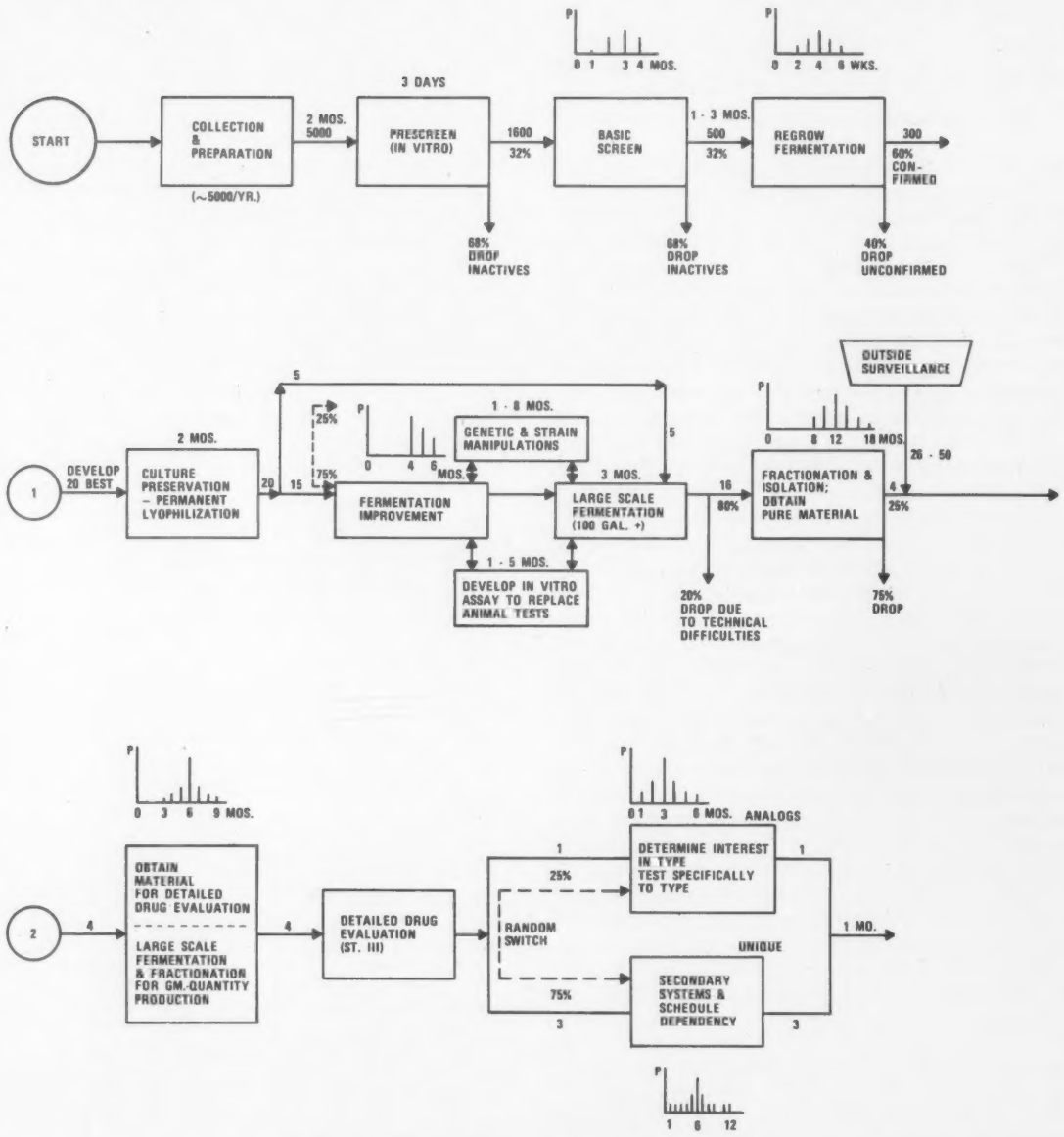


FIGURE 6.—Flow chart of the NCI's fermentation program.

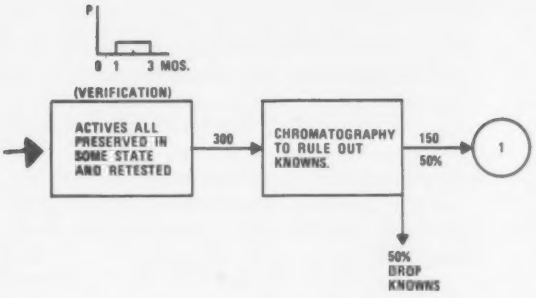
related genera have been studied for antibiotic production.

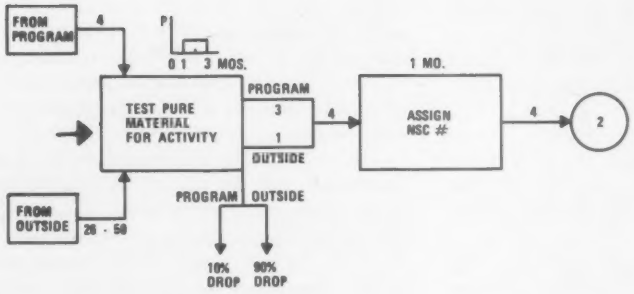
There is a very practical reason for this imbalance. In soil, streptomyces far outnumber the remaining Actinomycetales. For example, Lechevalier and Lechevalier (9) reported that of 5000 Actinomycetales isolated from soil, 95% were streptomyces. *Nocardia* represented only 1.98% of the isolated Actinomycetales, and all the others represented < 0.25% each. Thus, in a large screening program it is predictable that the majority of organisms tested will be streptomyces.[5]

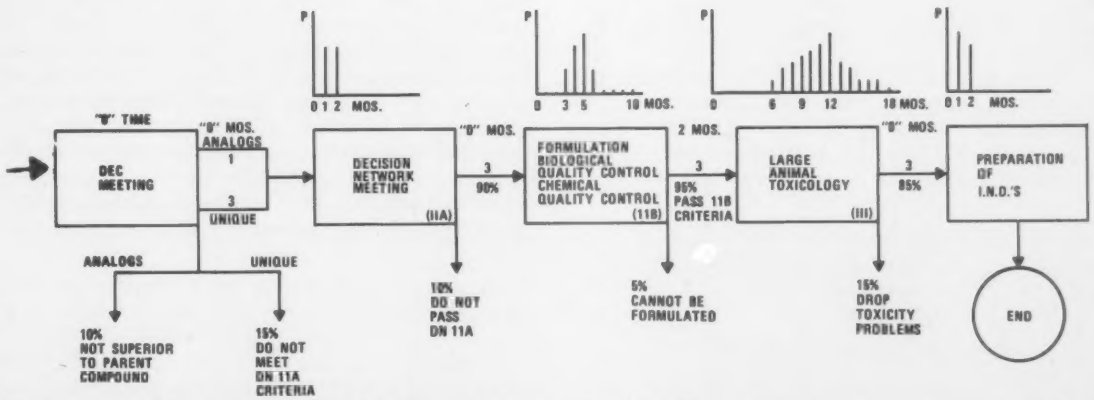
METHODOLOGY

The first step in the antibiotic program, the isolation (sprinkle plate, enrichment, percolation, etc) and purification of organisms, takes several months.

[5]*See* footnote 2.







We have recently incorporated substrates such as decane, hexadecane, and terpenes, to replace the common sugars used in isolation. We hope this will yield unique organisms not isolated in a normal fermentation program. Prior research indicates that a different microbial flora will utilize *n*-hexadecane than that which uses glucose. Many of the microbes that utilize hydrocarbons cannot assimilate carbohydrates, and the presence of glucose is inhibitory for many fermentation reactions (feedback repression) (10). For the most part, our contractors have used temperatures of 25°C–32°C, but in the future they wish to expand this to the psychrophilic and thermophilic ranges. Umezawa recently stated that many of his marine actinomycetes produce different metabolites when grown at 15°C compared to 25°C.[6] Anaerobes are now being evaluated for the first time in our program, but at present only for the

[6]*See* footnote 3.

biotransformation of various potential antineoplastic compounds.

Due to the time involved in testing fermentation broths for in vivo antitumor activity, it has been necessary to use several prescreening tests to determine which culture broths should be tested in animals as potential antineoplastic agents. Prescreens expedite the isolation of an antibiotic because an in vitro screen which correlates with in vivo activity can produce meaningful assay results much more rapidly. However, in vitro tests do not always correlate with in vivo activity and in some instances an animal screen must be used for all facets of the work. Using animal tests is quite time consuming and expensive but is necessary in order to obtain the active purified antineoplastic chemical.

The in vitro screens used today are KB (human epidermoid carcinoma of the nasopharnyx), P388 mouse leukemia (PS), L1210 mouse leukemia (LE), cell tissue culture toxicity tests, microbial assays, and some virus tests. This is an area the NCI hopes to expand to include some enzyme inhibition screens, viral tests, xenographs, and other biochemical tests.

Various research groups in Japan are using only enzyme systems as screens. The enzymes used are those which influence the DNA-RNA synthesis pathway, amino acid production, inhibition of ATP formation, etc. The NCI is sponsoring work to identify cell-cycle-specific agents (11) and has a contract with a group in Japan to evaluate enzyme inhibitor screens as indicators for potential antineoplastic agents (2).

After a fermentation broth, crude extract, or concentrate passes our in vitro tests it is tested in vivo against PS leukemia (fig 8). If it is active the culture is preserved. PS leukemia is used routinely to test antibiotics because of its sensitivity and ability to detect activity of clinically useful materials which are not active in the LE system. For example, mithramycin, a clinically active antibiotic, has definite activity in PS but minimal LE activity. Often an antibiotic is found in microgram or nanogram quantities per liter of fermentation broth, so that a screen sufficiently sensitive to prevent discarding potentially valuable agents is essential; the PS system appears to satisfy this requirement. Even so, many materials which might be of interest are discarded because the yield of the active material is too small to be detectable by the screens currently in use. To overcome the problems caused by low-yield fermentations, several of our contractors are testing various methods to concentrate the broth before testing for animal activity. This has increased the NCI's output of active fermentations in the few experiments evaluated. Lyophilization and solvent extraction are two methods used to concentrate broths which appear successful.

One of the NCI's newest candidates is an Upjohn compound, AT-125 (fig 9), which initially was not produced in sufficient yield to show activity in vivo. Due to a unique antimetabolite response (histidine reversed) which was identified in an antimetabolite screen, this material was extracted and found to be highly active in in vivo tests against PS and LE leukemias. This culture is now being fermented in large tanks so that sufficient AT-125 can be produced to evaluate formulations, carry out animal toxicology, and if interest continues, file an IND application and proceed to the clinic.

In our present scheme, if a fermentation culture produces an active substanee this activity must be reconfirmed at the same laboratory under identical conditions. Figure 6 shows 5000 microbial cultures initially screened, of which about 400 are active. At the reconfirmation step, 300 cultures at most are found to produce active antineoplastic materials. These cultures are preserved and chemical and

- Culture isolation (various soils worldwide)
  - Substrates – sugars, terpenes, hydrocarbons
  - Various temperatures
  - Aerobic, anaerobic
  - pH ranges
  - Media variations
  - Osmophiles, halophiles
  - Sprinkle plate, percolation, enrichment
- Purification
- Shake flask or tube fermentation
- Pre-screen
  (in vitro)
  - Cell toxicity
  - Antimicrobial activity
  - Antiviral activity

FIGURE 7.—Fermentation parameters evaluated.

Basic Screen

- PS in mice (crude broth, lyophilized, solvent extract of broth)
- Active ILS of > 25
- Shake flask fermentation

Reconfirm

- Fermentation produced under same conditions

Novelty Determinations

- Chromatography
- Bioautography
- Microbial spectrum

FIGURE 8.—Fermentation beers processed.

biologic tests are initiated to determine whether the material is of novel structure. It is essential to exclude known compounds from the list of active substances so as to more quickly isolate new compounds. The various chromatography identification steps eliminate at least 50% of the cultures because they are producers of known antineoplastic agents. Since the chromatographic tests are only presumptive and the actual material hasn't been isolated, it is possible to work on a known antibiotic because its chromatography and biologic results are masked by other materials in the fermentation broth. If the antitumor activity is better than that of the known material, work should continue since another active compound is present.

The danger always exists in a presumptive chromatography system that differences in structure are so subtle (actinomycin D vs actinomycin II, daunorubicin vs carminomycin vs adriamycin) that a new compound will be identified as a known, and that the new fermentation product will not be isolated and evaluated. The separation and identification of closely related compounds usually only take place during the isolation of active compounds. Unfortunately, no screen is capable of testing large numbers of fermentation broths so thoroughly as to obtain every possible lead.

Approximately 3% of the initial fermentations pass the chromatography stage although more recent prescreens have increased our productivity to about 20%. It normally takes 8 months for a culture to be isolated, reconfirmed as active in animal tests, and evaluated for novelty. It is at this point that approximately the top 20 fermentation cultures are selected for chemical fractionation. The selection is based on activity, uniqueness of culture, novelty determination, etc. The others are not discarded but are worked into the fractionation schedule when time permits.

It is rarely possible to be able to scale-up a fermentation from shake flasks to 5000-gallon tanks to obtain enough of the active component without doing fermentation-improvement studies. A typical fermentation medium which was obtained after optimization studies is shown in figure 10. This optimization of carbon, nitrogen, and mineral source concentrations along with the evaluation of environmental parameters (aeration, pH, CO_2, agitation, temperature, etc) is quite time consuming.

When a great deal of interest is generated in a substance because of its unique activity or structure, a genetic mutational program can be initiated to improve yield if fermentation-improvement studies fail. These programs normally take 6–8 months before sufficient fermentation batches can be supplied to the chemist for fractionation, isolation, and

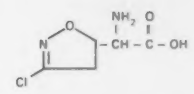


FIGURE 9.—Upjohn antineoplastic antibiotic AT-125.

1.5% glucose
1.0% soybean meal
0.5% peptone
0.5% meat protein
0.5% sodium chloride
0.5% dipotassium hydrogen phosphate, pH 6.8

FIGURE 10.—Typical fermentation medium (tubercidin production).

Fermentation Improvement

- Media
- pH
- Aeration (dissolved oxygen)
- Culture handling
- Temperature
- Time
- Precursors

Genetic & Strain Manipulations

- Strain selection
- Treatment with mutagenic agents
- Evaluation of different strains on mutants

FIGURE 11.—Techniques to improve antibiotic yields.

- Sufficient active material (30 - 3,000 gal.)
- Stability of material
- *In vitro* assay vs *in vivo* assay
- Molecule type - novel
- Concentration of material or materials

FIGURE 12.—Chemical fractionation and isolation.

- Enough pure material produced for stage III testing
  - Lewis Lung (IP & SC)
  - B16 (IP & SC)
  - L1210 (IC)
- Determine interest in structure type
- Secondary systems
- Dose responses

FIGURE 13.—Antibiotic stage III testing.

Source	Contract	Non-contract	Active
Actinomycetes	76	533	119
Fungi	12	179	23
Bacteria	–	42	9
Algae	–	5	–
Total	88	759	151

FIGURE 14.—Microbial types of fermentation products evaluated by the NCI.

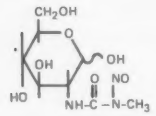


FIGURE 15.—Structure of streptozotocin.

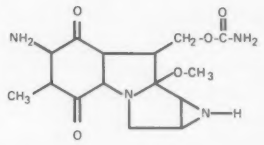


FIGURE 16.—Structure of mitomycin C.

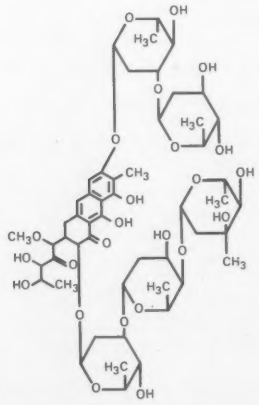


FIGURE 17.—Structure of mithramycin.

purification of the antibiotic (figs 11 and 12). Sometimes the programs are not successful and the lead will be discarded. Eighty percent of those cultures chosen for chemical work-up are improved enough for chemical fractionation and isolation of the active chemical. Twenty percent of the materials fractionated proceed to the Decision Network Meeting (DN2), the others may not be novel or may have problems of instability, purification, culture attenuation, etc.

One of the principal problems faced by the fermentation scientists is to increase antibiotic yields in large fermentors to permit the chemist to isolate enough material to determine structure and novelty, and to have enough material for thorough antineoplastic evaluation. The instability of the product is often a problem and causes a loss of activity. The speed with which a chemist can isolate the active compounds is usually governed by the concentration of the material in the broth, ability to find an in vitro assay which correlates with in vivo activity, and last but not least, stability. If the molecular type is novel to the program and active, the antibiotic receives top priority to expedite its testing (fig 12).

Figure 13 shows the type of testing which is done on an antibiotic after it has been purified enough to determine novelty and possibly its structure. We prefer pure compounds but proteins, glycoproteins, etc, are sometimes quite difficult to obtain in a pure state. We might also run additional special tests on the antibiotic such as tests to determine its activity against mammary and colon tumors and Ridgway osteogenic sarcoma as well as its animal toxicity.

Most antibiotics at this stage differ markedly in structure. However, those antibiotics which are analogs of compounds already evaluated at the NCI are compared with their counterparts, eg, actinomycin-like agents versus actinomycin D, or anthracyclines versus adriamycin. An antibiotic is handled like any other active chemical compound when it reaches DN2.

The NCI obtains many of its active antibiotics through its contract research process. Additional antibiotics are obtained for NCI evaluation through surveillance of the literature, worldwide industrial contacts, and correspondence with individual scientists. Although fewer compounds have come from contracts, these have almost all been active since through our fermentation scheme only materials which show in vivo activity are purified. The compounds obtained through surveillance of the literature are selected because of uniqueness of structure or some interesting biologic effect which may suggest the possibility of anticancer activity (fig 14).

Streptozotocin (fig 15).—This drug is a streptomyces-produced 2-methylnitrosourea derivative of glucose. Its mechanism of action has not been elucidated. The antibiotic is diabetogenic in animals but not in man. It is highly specific in its activity and is effective against functional malignant pancreatic islet cell tumors (12).

L-*Asparaginase.*—This is a protein which is produced by the bacterium *Escherichia coli (E. coli)*

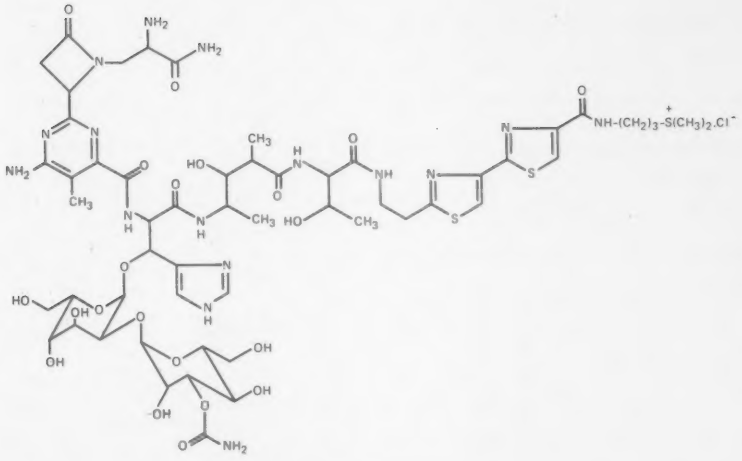


FIGURE 18.—Structure of bleomycin.

and catalyzes the hydrolysis of L-asparagine to aspartic acid and ammonia. Its mechanism of action is through the inhibition of protein synthesis. This enzyme is produced by *Erwinia*, *Proteus*, and other organisms, but only the *E. coli*-derived product has been clinically evaluated in the US. The antibiotic has been effective against acute lymphocytic leukemia (ALL).

Adriamycin (fig 2).—This drug differs from daunorubicin only by the presence of a hydroxyl group and it is produced by a mutant of the streptomyces that produces daunorubicin. It is effective against breast, ovary, and bladder cancers and some leukemias. Although it has cardiac toxicity it is probably the best antibiotic antineoplastic drug tested to date. Many aglycone compounds are being evaluated to determine whether they are active against adriamycin-resistant tumors and whether they maintain a high level of activity (12) without the severe cardiac toxicity seen with adriamycin.

Mitomycin C (fig 16).—This drug is a streptomyces-produced, fused heterocyclic compound containing an aziridine ring. As an alkylating agent it selectively inhibits DNA synthesis in organisms and is effective against myelogenous leukemia, reticulum-cell sarcoma, and breast and lung tumors.

RESULTS

The following antibiotics are considered clinically effective in cancer by the NCI (12):

Actinomycin D (fig 5).—This drug is a bright red and highly toxic polypeptide antibiotic produced by various species of streptomyces (12). Actinomycin D is effective in the treatment of Wilms' tumor (a rare cancer in the kidneys of children), testicular cancer, and some melanomas. Current therapy efforts with this drug are directed toward its utilization in combination with other active agents. Actinomycin D complexes with DNA which results in the inhibition of DNA-dependent RNA synthesis. The Drug Development Branch, Drug Research and Development Program, NCI, has tested 23 different actinomycins in the hope of obtaining an analog which is superior to actinomycin D. The results are not completed as yet.

Daunorubicin (fig 2).—This drug is a streptomyces-produced antibiotic. On acid hydrolysis this anthracycline aminoglycoside yields the aglycone daunomycinone and the sugar daunosamine (L-2-amino sugar). The L form of the sugars is rare. Daunorubicin in humans causes bone marrow suppression, but cardiac damage seems to be the major limitation of the drug. Daunorubicin is effective against granulocytic leukemia and ALL. Similar materials are being evaluated with the hope of finding one without cardiac toxicity but with antitumor activity (12).

Mithramycin (fig 17).—This drug is a streptomyces-produced polyhydroxylated, polycyclic hydrocarbon with a disaccharide and trisaccharide attachment. It inhibits DNA-directed RNA synthesis and is effective against testicular tumors. It is highly toxic and causes a hemorrhagic syndrome (12).

Bleomycin (fig 18).—This drug is a streptomyces-produced polypeptide antibiotic which inhibits DNA synthesis. It is active against squamous cell carcinoma of the head and neck, testicular tumors, and lymphomas. Umezawa found that bleomycin was

inactivated much more slowly in the skin and lungs than in other tissues, and predicted that it will be effective against skin epithelial cancer, Hodgkin's disease, and reticular sarcoma. Research in the Japanese laboratories continues to yield many different bleomycins which are frequently produced by adding various amines to the fermentations.

Several other antibiotics of interest to our program are as follows:

5-Methoxy-sterigmatocystin (fig 19).—This drug is an oxygen-containing, fused heterocyclic compound produced by the fungus *Aspergillus* and is similar to many aflatoxins. It is effective against several animal tumors (leukemia and solid tumors). However, the aflatoxins are highly toxic, causing liver and bladder disease.

L-Alanosine (fig 20).—This is a nitroso amino acid produced by a streptomyces and is effective against animal leukemias.

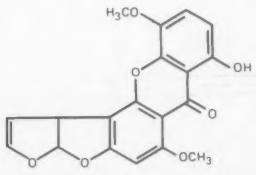


FIGURE 19.—Structure of 5-methoxy-sterigmatocystin.

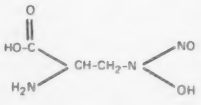


FIGURE 20.—Structure of L-alanosine.

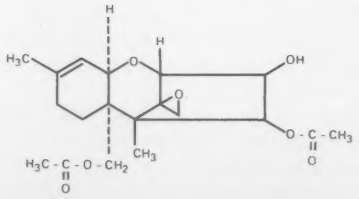


FIGURE 21.—Structure of anguidin.

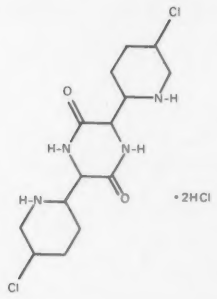


FIGURE 22.—Structure of piperazinedione.

- Mild Conditions
- One Step to Accomplish a Complicated Synthesis
- High Specificity
- Often Only Practical Synthesis

FIGURE 23.—Advantages of fermentation process.

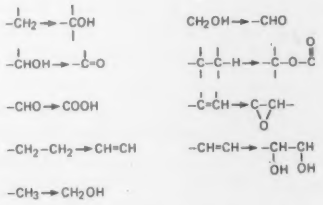


FIGURE 24.—Types of oxidation.

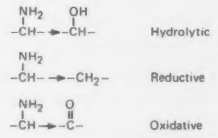


FIGURE 25.—Types of deamination.

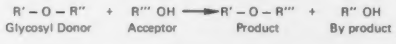


FIGURE 26.—Transglycosylation.

FIGURE 27.—Dismutation.

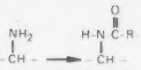


FIGURE 28.—Acylation.

- Rates of reaction are usually slow
- Batch equipment
- Influenced by many environmental changes (temperature, aeration, mineral concentration)

FIGURE 29.—Disadvantages of fermentation-type reactions.

- Systematic evaluation of microbial cultures fermented on various chemical compounds (purines, hydrocarbons, lignins)
- Evaluate psychrophiles, thermophiles, osmophiles
- Evaluate marine organisms, lichens
- Biotransformation and cometabolism of unusual compounds (antibiotics, plants, synthetic compounds)
- Enzymes as tumor inhibitors and as screening assay tools
- Mixed culture fermentation (Bleomycin producer & Adriamycin producer)
- Microbes as immunological stimulators
- Genetic manipulation of organisms
- Fermentation
  - Solid
  - Semi solid
  - Deep tank
  - Air lift
  - Continuous

FIGURE 30.—Future fermentation research possibilities.

Anguidin (fig 21).—This compound is a scirpinol (fused heterocyclic compound) with an epoxide ring and is produced by the fungus *Fusarium*. Anguidin is similar to several known mycotoxins and is scheduled for phase I clinical studies.

Piperazinedione (a substituted piperazinedione) (fig 22).—This drug is effective against animal leukemias.

Additional antineoplastic antibiotics including large and small molecular weight proteins, ansa type compounds, and peptides are undergoing extensive animal testing. Normally one to four new antibiotics per year are obtained from our contracts. These are novel and have been isolated and purified from fermentation broths.

COMMENTS

Earlier (fig 6) it was shown how leads progress through our fermentation scheme, and most readers are familiar with the pharmaceutic companies' usual approaches to finding antibiotics. In addition, we are currently substituting unique carbon sources for glucose (10) in the hope of producing new antibiotics. Biotransformation of steroidal starting materials indicated the possibility of doing the same with chemical molecules with anticancer activity which failed the NCI's criteria because of toxicity, insufficient activity, etc. Biotransformation and cometabolism fermentation research were added to the NCI's program but have not yet been explored in enough depth to determine the success of this type of research (5). Great pains have been taken to evaluate both known cultures and new cultures for their abilities to ferment purines, pyrimidines, hydrocarbons, terpenes, and other unique chemicals in addition to sugars.

There are certain advantages and disadvantages of a fermentation versus a chemical process. The advantages are listed in figure 23.

The microbe has the inherent ability to carry out at least 18 basic reactions (figs 4 and 24–28) through which it can build up a molecule, tear it down, or completely rearrange its structure in a manner which is impossible for the chemist to duplicate. The microbe often has the ability to manufacture a specific isomer. Often fermentation is the only practical method for producing sufficient quantities of a specific product such as an antibiotic or large molecular weight compound. Often a single compound is formed whereas a chemical reaction may give a mixture of compounds.

Inexpensive starting materials such as lignin can be used as the substrate. The microbes can utilize varied substrates as their carbon source, each of which influences the by-product formed by the organism. A fermentation process often requires only one step to accomplish a complicated synthesis. Mild conditions such as ambient temperature and atmospheric temperatures can be used which keep costs low.

The following are disadvantages of fermentation in comparison with chemical synthesis (fig 29). The rates of reaction are usually slow (conversion rates per volume handled are low). Today's fermentations are usually batch operations which inherently require longer down times and larger inventories than with a continuous unit. Such a fermentation operates only for a small percentage of the time at the optimal condition for the microbe, often only 10 minutes.

Fermentations are influenced by many environmental changes, ie, temperature, mineral concentration, aeration, etc, which can be the difference between making a product or not (citric or glutamic acids). The future of fermentation is great and undoubtedly many new chemotherapeutic agents will be discovered in the next decade. Figure 30 lists some of the potential areas to be explored. The program is in no way limited to these areas and

many of these may not be explored if more promising areas of potential new fermentation metabolites are uncovered.

The systematic evaluation of the various families of the microbial world is to determine their importance as antineoplastic, antibiotic producers. The evaluation of these cultures under various conditions, using carbon sources, is limited only by time and money. Judicious selection of substrates is essential to get the most mileage out of the available research dollars.

The NCI program has not yet explored the potential of psychrophiles, thermophiles, and osmophiles and it is conceivable that such unusual organisms might produce unusual compounds.

Very little fermentation work has been done to isolate and evaluate marine microorganisms and this might be of value. Several Japanese companies have indicated that novel biologically active materials have been obtained from marine fermentations.

Mixed culture fermentations may be of value as indicated by work done on chemicals produced by lichens. This indicates that a mixture of cultures produces different materials from any single culture. This type of work has many fermentation control problems but is worth investigation. The research on the immunologic response to cancer caused by using microbes of various types has potential. Genetic manipulation of organisms which can produce novel materials appears promising but mutant stability would be a problem. Various types of fermentations should be evaluated with organisms. The isolation of unique organisms is governed by the ingenuity of the microbiologist and thus this opens great possibilities of using unique chemicals as sole sources of carbon.

In conclusion I would simply like to quote the late Jackson Foster, University of Texas, "Never underestimate the power of the microbe."

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Actinomycin D: NSC-3053
L-Alanosine: NSC-529469; CAS reg. No. 16931-22-9
Anguidin: NSC-141537; CAS reg. No. 22135-66-6; spiro[2,5-methano-1-benzoxepin-10,2'-oxirane]-5α(6*H*)methanol, 2,3,4,5,7,9α-hexahydro-3,4-dihydroxy-5,8-dimethyl-, 4,5α-diacetate
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
Bleomycin: NSC-125066; CAS reg. No. 11056-06-7
Carminomycin: NSC-180024; 5,12-naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoxyl)oxy]-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy-, (8*S*-*cis*)-
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
AT-125: NSC-163501; 5-isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-
5-Methoxy-sterigmatocystin: NSC-178249; CAS reg. No. 50884-19-0
Mithramycin: NSC-24559; CAS reg. No. 18378-89-7
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7
Piperazinedione: NSC-135758; CAS reg. No. 41109-80-2; 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride
Streptozotocin: NSC-85998

REFERENCES

1. UMEZAWA H. Future prospect of antibiotics. Nippon Kagaku Ryohogakka Zasshi 22:1, 24:32, 1974.
2. ———. Enzyme Inhibitors of Microbial Origin. Tokyo, University of Tokyo Press, 1972.
3. MILLS KC, and LESSEL FLE. Designation and description of the type strain of *Lactobacillus casei* sub sp. a *lactosus* Rogosa et al. Int J Systm Bacteriol 23:67-68, 1973.
4. BRIAN PW. Antibiotics produced by fungi. Botanical Rev 17:357-430, 1951.
5. SEBEK OK. Microbial conversion of antibiotics. Lloydia 37:115-133, 1974.
6. STODOLA F, WALLEN LL, and JACKSON RW. Booklet ARS7-13 published by the Agricultural Research Service, USDA, Washington, DC, May 1959.
7. GORDON MH. A simulation study of the preclinical development of anticancer drugs. Cancer Chemother Rep Part 3, vol 5(1):65-78, 1974.
8. ROGOSA M, CUMMINS CS, LELLIOTT RA, ET AL. Actinomycetes and related organisms. *In* Bergey's Manual of Determinative Bacteriology, 8th ed. Baltimore, The Williams and Wilkins Co, 1974, pp 599-865.
9. LECHEVALIER HA, and LECHEVALIER MP. Biology of actinomycetes. Annu Rev Microbiol 21:71-100, 1967.
10. DONOVICK R, and BROWN WE. Comments on the general role of carbohydrates in antibiotic biosynthesis. *In* Biogenesis of Antibiotic Substances. Prague, Czechoslovak Academy of Sciences, 1965, pp 281-286.
11. LIPKIN M. Proliferative cycle of mammalian cells. *In* The Cell Cycle of Cancer (Baserga R, ed). New York, Marcel Dekker, 1971, pp 1-26.
12. LIVINGSTON RB, and CARTER SK. Single Agents in Cancer Chemotherapy. New York, IFI/Plenum, 1970.

Distribution of Anticancer Activity in Higher Plants

Arthur S. Barclay and Robert E. Perdue, Jr[1,*]

SUMMARY

The systematic distribution in higher plants of general anticancer activity, high-interest anticancer activity, and high-interest compounds is presented. Special emphasis is placed on those taxonomic groups offering, by virtue of their high-interest activity and/or compounds, the greatest potential as sources of useful anticancer agents.

[Cancer Treat Rep 60:1081–1113, 1976]

The search for higher plant sources of anticancer drugs began in 1957, initiated by the Cancer Chemotherapy National Service Center, now the Drug Research and Development Program (DR&DP), of the National Cancer Institute (NCI). Three years later, botanists of the Agricultural Research Service, US Department of Agriculture, were asked to participate in this research program by procuring plant materials for screening. This liaison was propitious because it brought botanical expertise, methodology, and philosophy into the screening program at an early stage of its development.

The objectives of the screening program are to test a broad spectrum of biologically active plant constituents in a group of selected, experimental, in vivo and in vitro tumor systems and to identify those of potential value for the treatment of cancer in man. For reasons described in other papers in this symposium and elsewhere (1–3), it was decided that a worldwide, random procurement program would best suit the objectives of the current large-scale, long-term search for plant sources of anticancer drugs. With few exceptions, broad screening, completely at random, has been the established *modus operandi*.

In keeping with the screening objectives, the botanists' primary role is the procurement of plant samples. The botanical input, however, does not end with the collection, documentation, and identification of these samples. It was realized from the outset that the systematic distribution of anticancer activity and active agents among higher plants could provide a scientific basis for selective plant procurement, once enough screening data were available. The purpose of this presentation is to define the state of the art, as of June 1975, in attaining this objective.

DEFINITIONS AND LIMITATIONS

In the context of this paper, "higher plants" are those that have developed vascular systems: the pteridophytes, or ferns and their allies, and the spermatophytes, or seed plants, which include the gymnosperms and the angiosperms. The data on anticancer activity in higher plants come from the files of the DR&DP, NCI, and are limited to experimental tumor systems. Frequent reference will be made to "active genera," those containing one or more "active species." "Distribution of anticancer activity" means the distribution of activity in recognized taxonomic groups such as genera, families, orders, and subclasses.

METHODS

Since its inception, the screening program has generated a wealth of data, positive and negative, on anticancer activity in higher plants and the types of agents responsible. It has passed through many stages of evolution, as outlined in other papers in this symposium. Not only has the screen continually evolved, but the preparation of crude extracts for testing has changed also—the most recent during the last year when a preliminary fractionation procedure was adopted to concentrate

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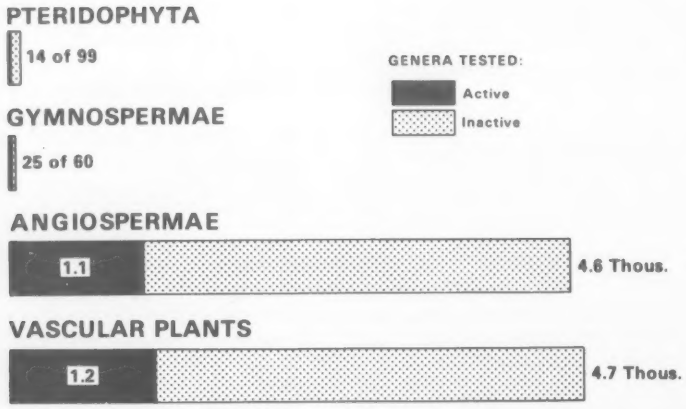

FIGURE 1.—Nos. of genera screened and active in the 3 major divisions of higher plants and in the higher (vascular) plants as a whole.

active agents before testing (4). All of these changes have a profound impact on the yield of anticancer activity and active agents.

As screening methods evolved, so did the significance of anticancer activity. Concomitantly, the accuracy of the screen in predicting potential sources of anticancer drugs increased. Earlier reviews of screening (3,5) revealed not only high concentrations of activity in certain plant families, but activity diffused among many families. It is now evident that this broad spectrum is due to the great chemical diversity of plant products capable of inhibiting cancer growth in laboratory animals. Furthermore, a high percentage of these plants owed their efficacy to tannins and phytosterols, nearly ubiquitous in higher plants, which have no real value as anticancer agents. The primary screen, a result of much experimentation, now consists of two tumor systems insensitive to tannins and phytosterols: a cell culture of human epidermoid carcinoma of the nasopharynx (KB) and mouse P388 lymphocytic leukemia (P388).

As used in this paper, anticancer activity includes effectiveness against all tumor systems, both current and obsolete. With general activity being so variable, it is evident that merely documenting its distribution in higher plants will not provide solid leads to sources of potentially useful anticancer agents. However, valuable clues can be obtained if close attention is given to activity against the in vivo tumor systems most likely to be predictive for clinical activity in man: namely, P388 and mouse L1210 lymphoid leukemia (L1210). When these data are combined with what is known about the nature of active compounds isolated from plants and incorporated into a reliable botanical classification, true correlations emerge among activity, compounds, and related plant groups.

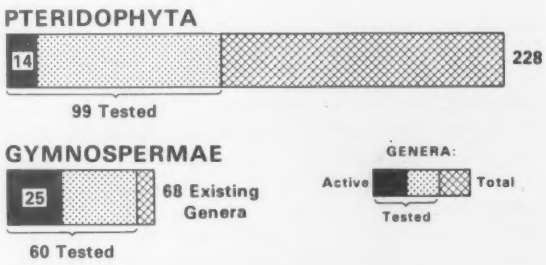

FIGURE 2.—Nos. of active, tested, and total genera in the Pteridophyta and Gymnospermae.

This paper will first discuss the distribution of anticancer activity in the major categories of higher plants, and will then narrow down to the most promising classes, subclasses, orders, and families. To bring the most important sources of potential anticancer agents into bold relief, three high-interest criteria were selected: (*a*) the five most important classes of chemical compounds;[2] (*b*) activity against L1210; and (*c*) high T/Cs in P388.[3] The

[2]Many varieties of compounds with anticancer activity have been isolated from higher plants. In this symposium, Hartwell (1) has identified those compounds belonging to five chemical classes as being of greater interest than the others: diterpenes, lignans, quassinoids, ansa macrolides, and alkaloids.

[3]In the two leukemia systems, no distinction is made between marginal and high activity except in the case of P388. The degree of activity in P388 is determined by the T/C or ratio of average survival of treated animals in days compared to that of controls × 100. While a T/C of 125% is an indication of activity, a figure of 175% is usually required for a priority high enough to warrant advanced screening (1). In the context of this paper, a high T/C in P388 is ≥ 175%.

resulting systematic distribution of these criteria among higher plants can provide guidance to productive plant procurement and screening.

SCOPE AND IMPLICATIONS OF DATA

Overall Extent of Anticancer Screening and Yield of Anticancer Activity in Higher Plants

Any comprehensive consideration of the distribution of anticancer activity in higher plants should take into account the proportion of plants screened to those not screened. More than 67,500 plant extracts have been screened, representing 20,525 species in 4716 genera and 317 families. Based on recent estimates,[4] about 9% of the species, 39% of the genera, and 83% of the families of higher plants have been screened.

Of the 67,500 extracts tested, 2787 have shown confirmed activity against one or more of the tumor systems used for screening since the inception of the program. The active extracts represent 2127 species in 1225 genera and 189 families. About 4% of the extracts, 10% of the species, and 26% of the genera submitted for screening were active.

Comparison of Anticancer Activity in the Major Groups of Higher Plants[5]

Figure 1 illustrates the number of genera screened and found to be active in the higher (vascular) plants as a whole and compares the number of genera screened and determined to be active in the pteridophytes, gymnosperms, and angiosperms. The Angiospermae far outweigh the Pteridophyta and Gymnospermae in numbers of tested and active genera. This is due to the sizes of the three groups; together, the pteridophytes and gymnosperms contain an estimated 296 genera, whereas the angiosperms contain 11,680 genera.

The number of tested, active, and total genera in the pteridophytes and gymnosperms is indicated in figure 2. In the pteridophytes, only 14% of the genera tested were active, whereas in the gymnosperms, 42% of the genera tested were active. The percentage of active genera in the gymnosperms is well above the 26% yield in the higher plants as a whole and three times greater than that of the pteridophytes.

So far, the pteridophytes have yielded practically no activity against the tumor systems currently in use. Only one fern, *Jamesonia imbricata* var. *glutinosa* (Karst.) A. Tryon, has shown activity against P388. The remaining active pteridophytes were active only in the obsolete, tannin-sensitive tumor systems. Indeed, the only active agents isolated from the pteridophytes are tannins. Hence, the ferns and their allies are eliminated from further consideration here. The more sophisticated preliminary fractionation prior to screening (4) may reveal promising activity in the pteridophytes, but this remains to be seen.

Among higher plants, truly promising activity and compounds have been found only in the spermatophytes. Therefore, the remainder of this presentation will concentrate on this group which constitutes the majority of dominant land plants: the gymnosperms and the angiosperms.

In terms of total genera and species available for screening, the gymnosperms are only a fraction the size of the angiosperms: 759 species in 68 genera and 12 families versus 220,000 species in 11,680 genera and 340 families. Nevertheless, the gymnosperms (table 1) are nearly twice as productive as the angiosperms in the yield of active extracts, species, and genera. However, the activity (table 1)

TABLE 1. – SCREENING AND ACTIVITY IN SPERMATOPHYTES

	Gymnospermae	Angiospermae
Extracts		
No. tested	2,164	64,634
No. active	166	2,602
% activity	8%	4%
Species		
No. tested	393	19,796
No. active	88	2,022
% activity	22%	10%
Genera		
No. tested	60	4,557
No. active	25	1,066
% activity	42%	23%
Families		
No. tested	11	285
No. active	8	175
% activity	73%	61%

[4]In this paper, the numbers of species and genera in the families, orders, subclasses, classes, and divisions of the higher plants are based on estimates from several sources (6–9).

[5]Throughout this paper, genera are used to illustrate comparative anticancer activity, since they, more than species, indicate the degree of morphologic and chemical diversity within taxonomic groups of plants at or above the family level.

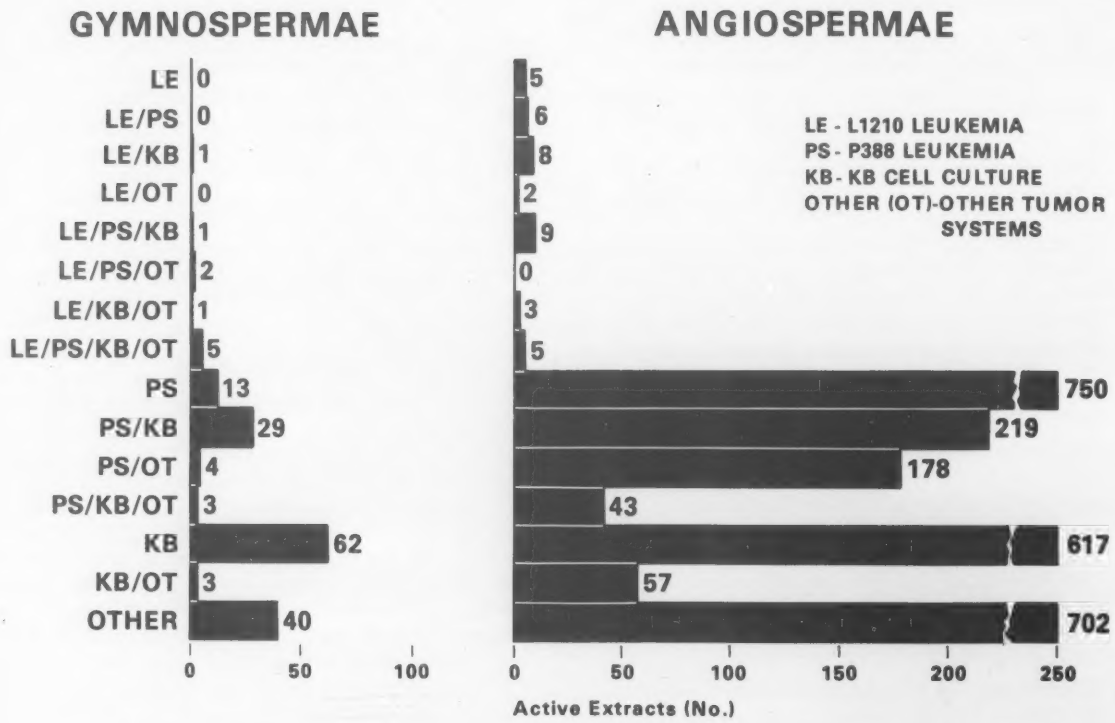

FIGURE 3.—"Activity profiles" for the Angiospermae and Gymnospermae. *See* text for further details.

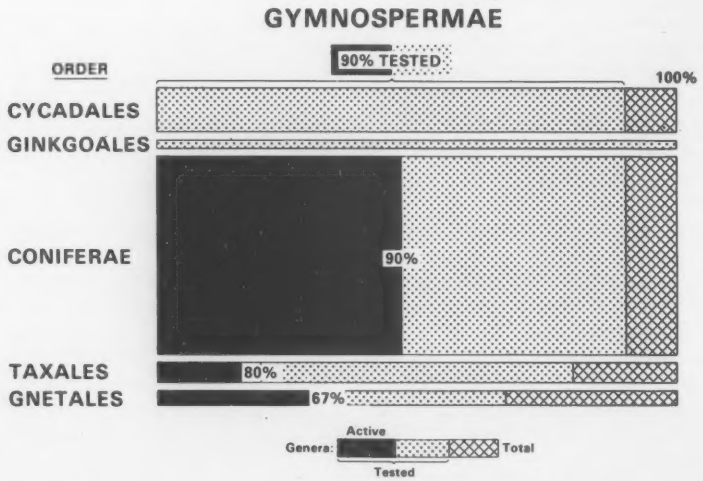

FIGURE 4.—Extent of screening and comparative yields of anticancer activity in orders of Gymnospermae. The 5 orders of Gymnospermae are represented by horizontal bars. The depth of each bar, determined by the number of genera in the orders, is a measure of their relative size and diversity. The length represents 100% of the genera in each order. The combined solid and stippled portions indicate the percentage of genera tested in the orders, whereas the crosshatched portion indicates the percentage of genera not yet screened. The solid portion of the bar represents only the percentage of activity in the genera tested, not a percentage of the whole.

is the general activity accrued against all tumor systems, current and obsolete.

To gain a perspective of the amount of potentially useful activity in the gymnosperms and angiosperms, it is worthwhile to examine the distribution of activity in the tumor systems used in the screen, with major emphasis placed on those currently in use. For this purpose, "activity profiles" were prepared (*see* fig 3) for both gymnosperms and angiosperms. In these, the obsolete systems discussed in other papers of this symposium (1,10) appear in the "other" category. As shown in figure 3, the screen consists of KB, P388, L1210, and other. There are 15 possible combinations of these. By definition, an "active extract" must be effective against one or more of the systems represented.

There is one striking difference between the two "activity profiles" (fig 3). In the Gymnospermae, activity is proportionally highest in KB, while in the Angiospermae, activity is highest in P388. The high KB activity of the gymnosperms is probably due to the presence of cytotoxic lignans, to which KB is sensitive.

In both the Gymnospermae and Angiospermae, there are significant numbers of extracts active in both KB and P388, and the chances are good that many of these will prove to be active against L1210. Active compounds isolated from these plants will later be evaluated in the slow-growing solid tumors: mouse B16 melanoma and the new mouse Lewis lung carcinoma (LL). Those that are active in B16 or LL will become strong candidates for preclinical evaluation.

Anticancer Activity in Orders and Families of the Gymnospermae

Figures 4 and 5 illustrate the extent of screening and comparative yields of activity in the orders and families of the Gymnospermae as recognized in the 12th edition of *A. Engler's Syllabus der Pflanzenfamilien* (8).

Figure 4 depicts the relative sizes of the five orders of Gymnospermae. The percentage of genera tested is numerically indicated and the solid portion represents the percentage of these found active.

Only three orders are of significance. One of these, the Gnetales, has little activity in the important tumor systems.

Figure 5 narrows the field to families of the two orders having significant activity: (*a*) the Coniferae containing the families Pinaceae, Taxodiaceae, Cupressaceae, Podocarpaceae, Cephalotaxaceae, and Araucariaceae; and (*b*) the Taxales containing the single family Taxaceae. The left bar graph illustrates the number of active, tested, and total genera in each family. These families have been intensively screened and each contains active genera.

The right bar graph shows the percent of active extracts, a good indicator of promising groups. This points up three attractive families: the Cupressaceae, Cephalotaxaceae, and Taxaceae. In fact, these are the only families in the Gymnospermae having L1210 activity (table 2).

Distribution of High-Interest Activity and Compounds in Families of the Gymnospermae

In this section, the high-interest activity and compound criteria referred to earlier will be applied to further narrow the field to those families with the greatest potential as sources of anticancer agents.

In the Gymnospermae, high-interest compounds have been isolated from 15 species in eight genera and five families (table 2). Among these compounds are three alkaloids included in Hartwell's list (1) of the 21 most interesting compounds isolated from plants: harringtonine, homoharringtonine, and taxol. Also, ten species in five genera and three families are active against L1210 and seven species in five genera and four families have high T/Cs in P388 (table 3).

These selected criteria identify at least three families of special interest: the Cephalotaxaceae, Cupressaceae, and Taxaceae. Though of less interest, the Podocarpaceae and Taxodiaceae are promising families worthy of further exploration.

Although the Gymnosperms have been an important source of potentially valuable compounds, the possibilities of locating new active compounds would appear to be low because few genera remain to be screened (cf, figs 4 and 5). Nevertheless, the new extraction procedure (4) may reveal additional novel compounds of key interest in those genera already tested and found inactive.

Roughly 88% of the genera and 52% of the species of Gymnospermae have been screened. In comparison, only 39% of the genera and 9% of the species of Angiospermae have been screened. The Angiospermae contain, by far, the greatest number of active genera and species and have been the source of most of the high-interest compounds. They are clearly the more promising group for future screening.

Extent of Screening and Activity in the Classes, Subclasses, and Orders of the Angiospermae

The remainder of this paper will focus on the distribution of anticancer activity and high-interest

TABLE 2.—HIGH-INTEREST COMPOUNDS IN GYMNOPERMAE

COMPOUND	GENUS AND SPECIES	FAMILY
Alkaloids		
Harringtonine	*Cephalotaxus harringtonia* (Knight ex Forbes) K. Koch	Cephalotaxaceae
Homoharringtonine	" " " " " " "	"
Isoharringtonine	" " " " " " "	"
Methoxyharringtonine	" " " " " " "	"
Taxol	*Taxus brevifolia* Nutt.	Taxaceae
	T. canadensis Marsh.	"
	T. cuspidata Sieb. & Zucc.	"
	T. X *media* Rehd.	"
a Tetraol	*T. brevifolia*	"
Diterpenes		
Podolide	*Podocarpus gracilior* Pilg.	Podocarpaceae
Taxodione	*Taxodium distichum* (L.) Rich.	Taxodiaceae
Taxodone	" " " "	"
Lignans*		
Deoxypodophyllotoxin	*Callitris columellaris* F. Muell.	Cupressaceae
	Calocedrus decurrens (Torr.) Florin	"
	Juniperus communis L.	"
	Thuja occidentalis L.	"
Podophyllotoxin	*Juniperus chinensis* L.	"
	J. virginiana L.	"
Podophyllotoxin glucoside	*Callitris drummondii* (Parl.) F. Muell.	"

*Until recently, the lignans were considered to be low interest types of compounds; podophyllotoxin and several related natural products proved to be of little clinical interest. However, their status has been changed to the high interest category because several semi-synthetic derivatives have shown some clinical activity against brain tumors, lymphosarcomas and Hodgkin's disease (1).

TABLE 3.—HIGH-INTEREST ACTIVITY IN GYMNOSPERMAE

FAMILY, GENUS AND SPECIES	T/C in P388 ≥ 175%	L1210 Activity
Cupressaceae		
Chamaecyparis lawsoniana (A. Murr.) Parl.		+
Cupressus lusitanica Mill.	+	
Juniperus chinensis L.	+	+
J. communis L.		+
J. scopulorum Sarg.		+
Thuja occidentalis L.		+
Podocarpaceae		
Podocarpus gracilior Pilg.	+	
P. milanjianus Rendle	+	
Cephalotaxaceae		
Cephalotaxus harringtonia (Knight ex Forbes) K. Koch	+	+
Cephalotaxus wilsoniana Hayata	+	
Taxaceae		
Taxus baccata L.		+
T. brevifolia Nutt.		+
T. canadensis Marsh.		+
T. cuspidata Sieb. & Zucc.		+
T. X media Rehd.	+	

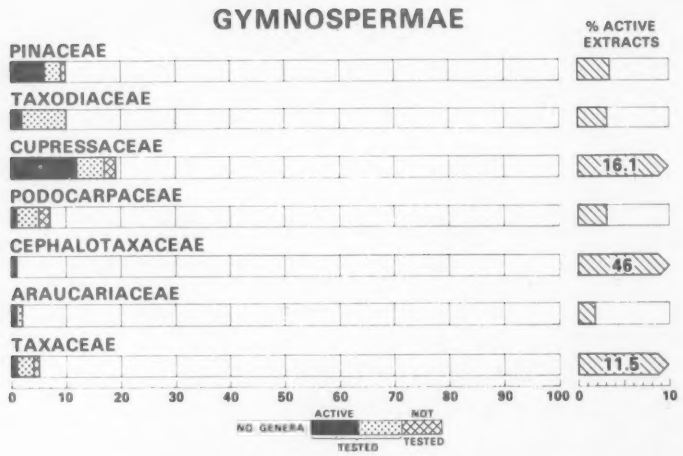

FIGURE 5.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in families of Coniferae and Taxales.

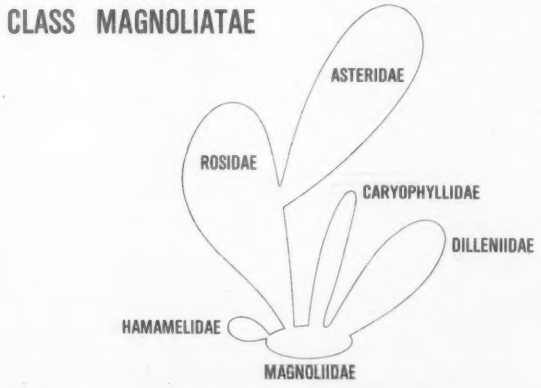

FIGURE 6.—Phyletic diagram illustrating Cronquist's (7) conception of the probable relationships among the 6 subclasses of Magnoliatae. The size of each balloon is proportional to the number of species in each subclass. [Redrawn with slight modifications from Cronquist (7)].

TABLE 4.—SCREENING AND ACTIVITY IN CLASSES OF ANGIOSPERMAE

	Magnoliatae	Liliatae
Extracts		
No. tested	59,640	4,994
No. active	2,366	236
% activity	4%	5%
Species		
No. tested	17,829	1,940
No. active	1,837	185
% activity	10%	10%
Genera		
No. tested	3,950	607
No. active	956	110
% activity	24%	18%
Families		
No. tested	242	43
No. active	157	18
% activity	65%	42%

compounds in the Angiospermae. This, and the following discussions of the Angiospermae, will draw heavily on the system of classification outlined by Arthur Cronquist in his book, *The Evolution and Classification of Flowering Plants* (7).

In Cronquist's system of classification, the Magnoliophyta or Angiospermae are divided into two classes: the Magnoliatae or dicots, and the Liliatae or monocots. According to his estimates, the Liliatae are only a third the size of the Magnoliatae: 55,000 species *versus* 165,000 species. The majority of the Angiospermae screened belong to the Magnoliatae (table 4). The two classes are nearly equal in percentages of active extracts and species, but the percentages of active genera and families are significantly higher in the Magnoliatae. The reasons for this will be apparent later.

Figures 6 and 7 illustrate Cronquist's concepts of the probable relationships among the subclasses of the Magnoliatae and Liliatae. In both figures, the

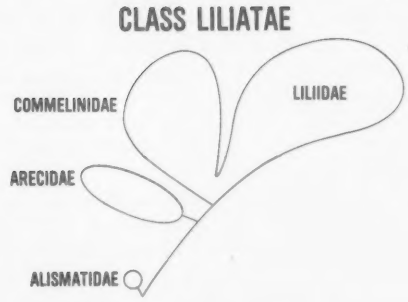

FIGURE 7.—Phyletic diagram illustrating Cronquist's (7) conception of the probable relationships among the 4 subclasses of Liliatae. The size of each balloon is proportional to the number of species in each subclass. [Redrawn with slight modifications from Cronquist (7)].

size of each "balloon" is proportional to the number of species in the subclass. It is not apparent from these diagrams, but according to Cronquist, "The Magnoliidae are the basal complex from which all other angiosperms have been derived."

Figures 8–17 show the relative sizes of the principal orders of the ten subclasses. The percentage of genera tested is numerically indicated and the solid portion represents the percentage of these found active. The orders and their sequence in the graphs follow Cronquist's system of classification. In some of the graphs, one or more small orders have been excluded.

Class Magnoliatae

Subclass Magnoliidae (11,000 species in 473 gen-

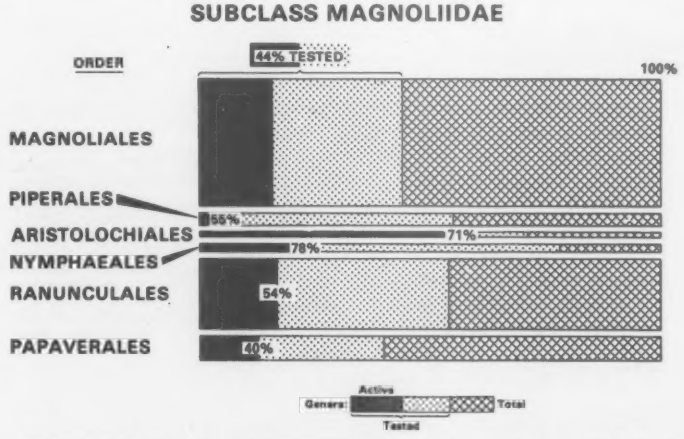

FIGURE 8.—Extent of screening and comparative yields of anticancer activity in the 6 orders of Magnoliidae. *See* fig 4 for explanation and interpretation.

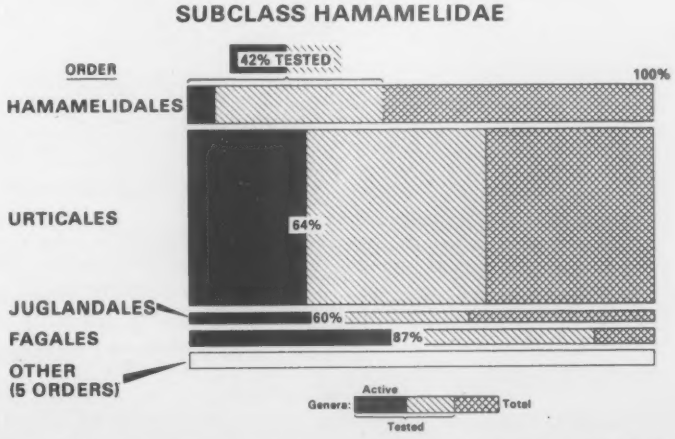

FIGURE 9.—Extent of screening and comparative yields of anticancer activity in 4 of the 9 orders of Hamamelidae. *See* fig 4 for explanation and interpretation.

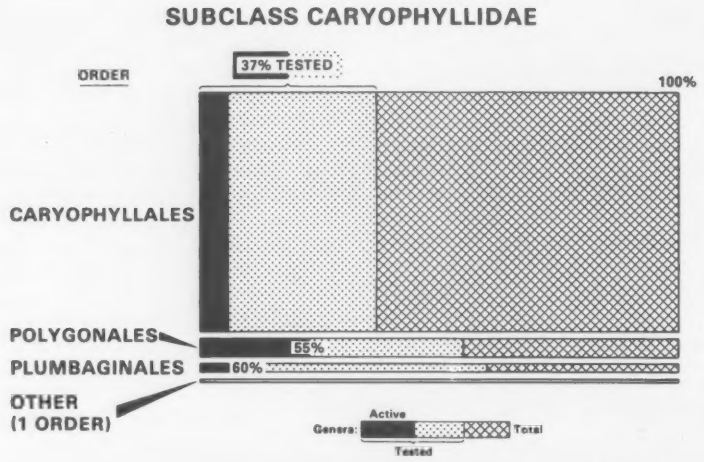

FIGURE 10.—Extent of screening and comparative yields of anticancer activity in 3 of the 4 orders of Caryophyllidae. *See* fig 4 for explanation and interpretation.

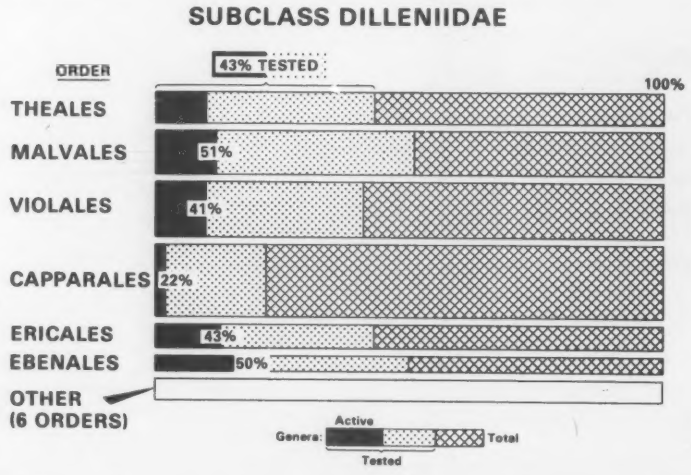

FIGURE 11.—Extent of screening and comparative yields of anticancer activity in 6 of the 12 orders of Dilleniidae. *See* fig 4 for explanation and interpretation.

era, 34 families, and six orders).—All orders of this primitive subclass have been screened (fig 8) and activity has been found in each. With the exception of the Piperales, the percentage of active genera in these orders is above the 26% yield for active genera in the higher plants as a whole: 36% for the Magnoliales, 80% for the Aristolochiales, 29% for the Nympheales, 33% for the Ranunculales, and 32% for the Papaverales. Also, high-interest compounds and activity have been found in four of the six orders.

Subclass Hamamelidae (3400 species in 184 genera, 21 families, and nine orders).—In this smallest subclass of the Magnoliatae, representatives of six of the nine orders screened were found active. Two of the inactive orders, the Eucommiales and Leitneriales, are monotypic, and the third, the Trochodendrales, contains only two species. The more important orders of the subclass are included in figure 9. The orders in this subclass have been broadly screened, and a substantial number of genera are active. However, the only valuable activity and compounds are concentrated in the Urticales. This order contains nearly 70% of the genera in the subclass.

Subclass Caryophyllidae (11,000 species in 554 genera, 17 families, and four orders).—Although activity occurs in three of the four orders tested (fig

10), the active compounds were found to be tannins, phytosterols, saponins, and proteins, all of little interest. Although an active alkaloid was isolated from one cactus species, this relatively small subclass seems to offer little promise.

Subclass Dilleniidae (24,000 species in 1437 genera, 72 families, and 12 orders).—The principal orders in this subclass are represented in figure 11. Activity was found in all but the Diapensiales, but the active compounds isolated so far are mainly tannins, phytosterols, and cucurbitacins which now are of no interest.

Subclass Rosidae (60,000 species in 3140 genera, 100 families, and 16 orders).—The most important orders in this largest subclass of the Magnoliatae are included in figure 12. Two of the smallest, the Podostemonales and Rafflesiales, have not yet been screened, but of the 14 tested, activity was found in all but the Haloragales. Although the orders in figure 12 appear to have activity similar to that shown in the preceding figures, these orders are much more promising because of the types of active compounds isolated.

Subclass Asteridae (56,000 species in 3325 genera, 43 families, and nine orders).—Anticancer activity has been found in all nine orders (fig 13). In contrast to the other subclasses of the Magnoliatae, the Asteridae, at the evolutionary pinnacle of the dicots, are derived from the Rosidae (*see* fig 6). Like the Rosidae, they are a rich source of high-interest compounds and activity.

It is evident that anticancer activity is nearly ubiquitous among the dicots: 54 of the 56 orders have been screened and anticancer activity has been found in all but six, each of which is very small.

Class Liliatae

Subclass Alismatidae (500 species in 55 genera, ten families, and four orders).—Although one order remains to be screened, no activity has been found in the chiefly aquatic Alismatidae (fig 14).

Subclass Commelinidae (19,000 species in 960 genera, 20 families, and eight orders).—The five principal orders in this subclass are depicted in figure 15. With the exception of the Eriocaulales, activity was found in all orders. However, the percentage of active genera in the subclass is only 13%, with most of the activity attributable to tannins. Because they contain the grass family, the Commelinidae are one of the most important groups of economic plants. However, they appear to offer little hope as sources of anticancer drugs. Botanists involved in the plant screening program commonly refer to a promising plant family as a FOSI, or *family of special interest;* those of no interest are described by the acronym FONI, or *family of no interest*. Past screening experience relegates the grass family to the FONI category. In fact, the grasses show so little promise that they were the first family so designated. The orders of the Commelinidae follow the pattern of the grass family.

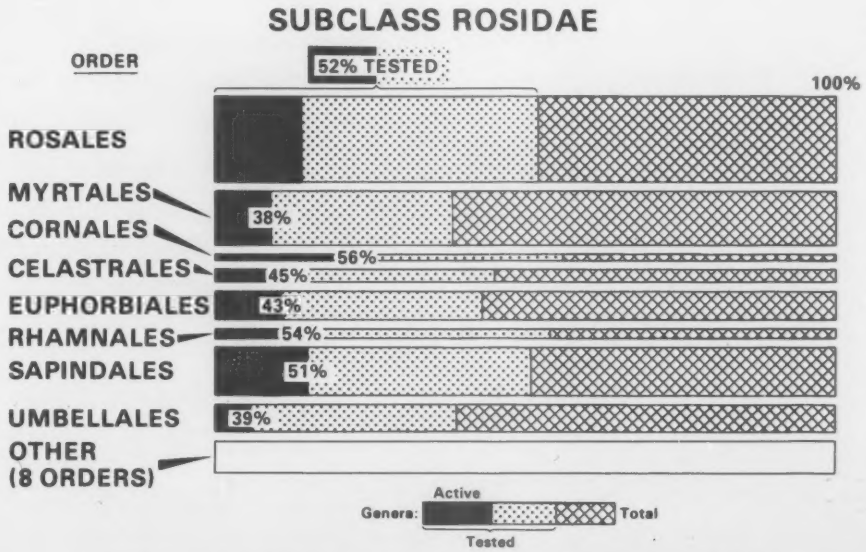

FIGURE 12.—Extent of screening and comparative yields of anticancer activity in 8 of the 16 orders of Rosidae. *See* fig 4 for explanation and interpretation.

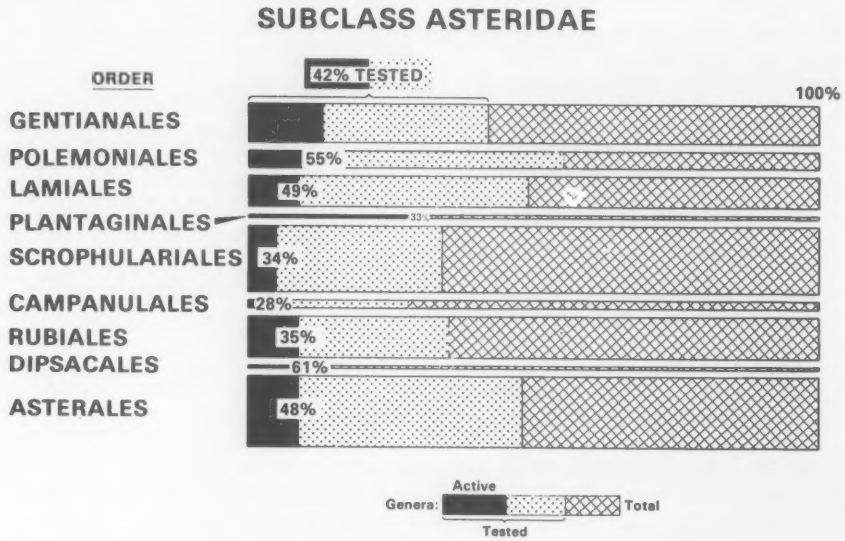

FIGURE 13.—Extent of screening and comparative yields of anticancer activity in the 9 orders of Asteridae. *See* fig 4 for explanation and interpretation.

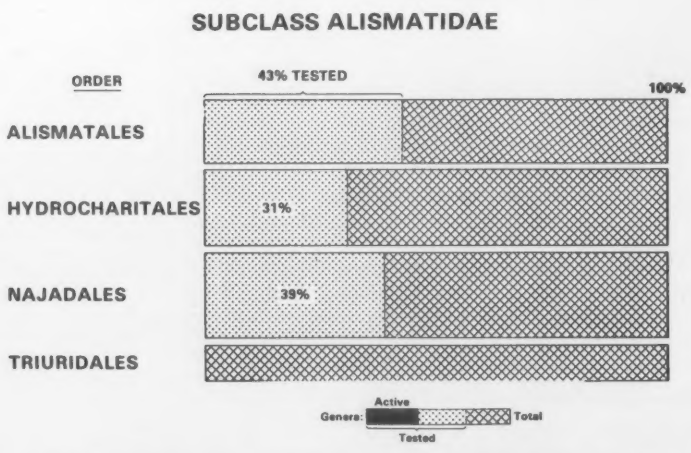

FIGURE 14.—Extent of screening and comparative yields of anticancer activity in the 4 orders of Alismatidae. *See* fig 4 for explanation and interpretation.

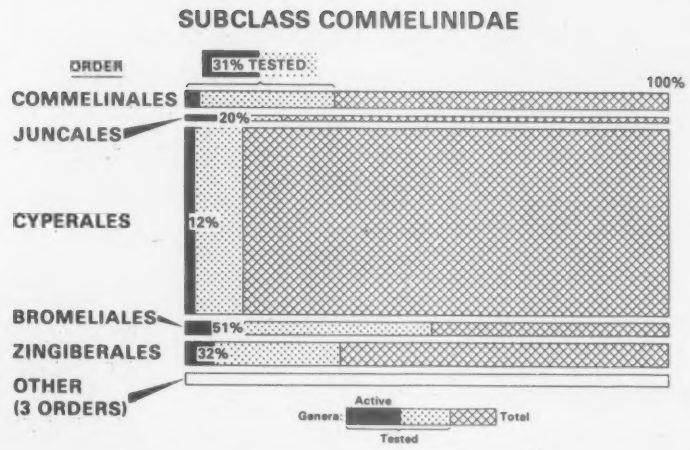

FIGURE 15.—Extent of screening and comparative yields of anticancer activity in 5 of the 8 orders of Commelinidae. *See* fig 4 for explanation and interpretation.

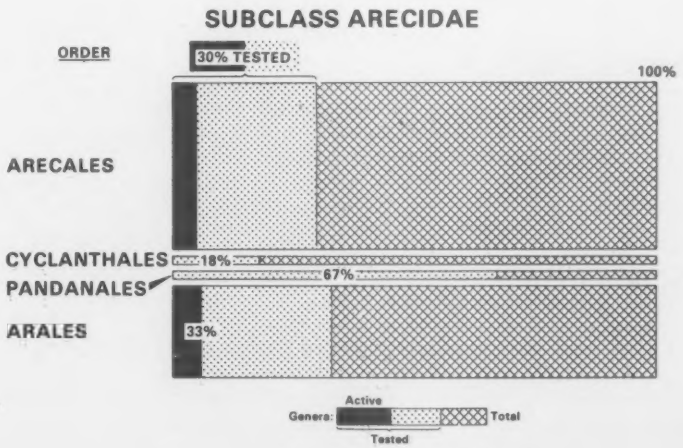

FIGURE 16.—Extent of screening and comparative yields of anticancer activity in the 4 orders of Arecidae. *See* fig 4 for explanation and interpretation.

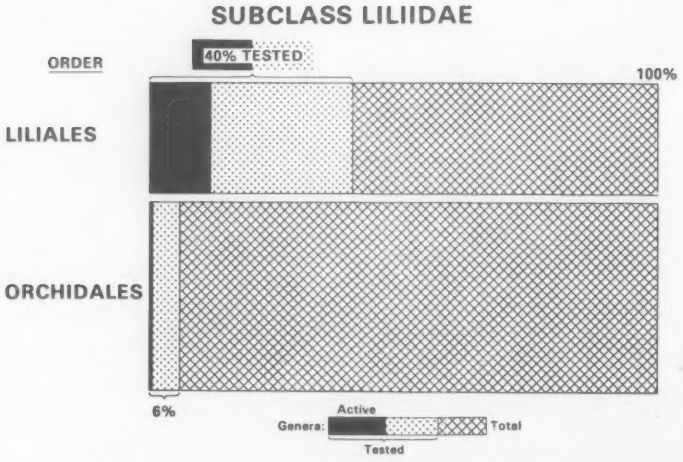

FIGURE 17.—Extent of screening and comparative yields of anticancer activity in the 2 orders of Liliidae. *See* fig 4 for explanation and interpretation.

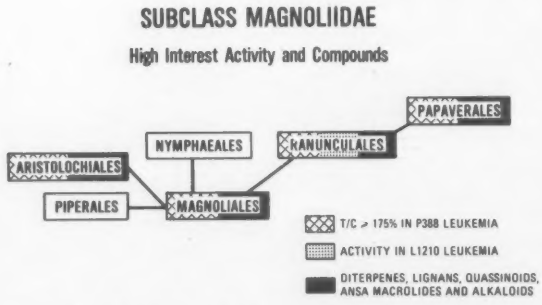

FIGURE 18.—Distribution of high-interest activity and compounds among the orders of Magnoliidae. Here, and in the following 4 figs, the phyletic diagrams illustrate Cronquist's (7) concepts of the probable relationships among the orders of the subclasses. Also, according to Cronquist, "the length of the line between two orders is governed by the requirements of two-dimensional representation and does not indicate the closeness of the relationship." [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

Subclass Arecidae (6400 species in 352 genera, five families, and four orders).—This subclass includes the palms, aroids, and screwpines (fig 16). Activity is present in two of the four orders, but the only active compounds isolated from the entire group are tannins.

Subclass Liliidae (28,000 species in 1200 genera, 18 families, and two orders).—The family Orchidaceae, containing an estimated 17,000 species in 735 genera, makes the Orchidales the larger of the two orders (fig 17). Although they are one of the largest families of flowering plants, rarely is any one species abundant enough to provide adequate samples for screening. Consequently, the Liliales have received the greatest attention, proving to be an excellent source of high-interest compounds and promising activity. Among the monocots, they hold the greatest promise for future screening.

Distribution of High-Interest Activity and Compounds in the Subclasses and Orders of the Angiospermae

It is apparent from the preceding discussion and figures that general anticancer activity, without additional criteria, will not provide leads to the most promising sources of anticancer drugs. Subclasses appearing to have a fair amount of activity can be of low interest because the plants tested lack activity against the current in vivo tumor systems, or because the active compounds isolated are not of interest.

Obviously, the potential of taxonomic groups will rise or fall on the basis of the compounds they provide. However, the agents responsible for the activity in many plants are unknown. Consequently, program guidance must also rely on high-interest activity.

In this section, the three high-interest criteria previously applied to the gymnosperms will again be applied to identify those subclasses and orders with the most potential. A combination of these criteria will reinforce those taxonomic groups already known to be important sources of compounds, and should indicate where to look for new sources of novel, high-interest compounds.

The following discussion and figures will continue to draw heavily on Cronquist's system of classification (7). The phyletic diagrams used in the following

figures were designed by him to express his concepts of probable relationships among the orders of the subclasses. These have proved particularly adaptable to the illustration of the distribution of high-interest compounds and activity.

The discussion following this one will narrow down to the families in orders identified by the three criteria.

Class Magnoliatae

Subclass Magnoliidae.—Figure 18 illustrates the distribution of high-interest compounds and activity among the orders. Two important types of compounds have been isolated, lignans and alkaloids; their occurrence in orders of the Magnoliidae is shown in table 5. Lignans occur in three related orders, including two that also occur in the gymnosperms: podophyllotoxin and deoxypodophyllotoxin. Active alkaloids have also been isolated from members of three orders following the chain of evolution at approximately two o'clock in the diagram (fig 18). They include thalicarpine and liriodendine in the Magnoliales, thalicarpine and berberine in the Ranunculales, and berberine in the Papaverales. Species with high T/Cs in P388 occur in four orders, and two species in the Ranunculales have activity against L1210. The bioassay criteria have identified no new orders of interest and the Piperales and Nymphaeales appear to offer little promise for the future.

Subclass Hamamelidae.—There is a broad spectrum of activity among the orders of this subclass (*see* fig 9), due almost entirely to tannins, occurring here in great abundance, and phytosterols. Only in the order Urticales is there any promise for the future (fig 19): the active alkaloid cryptopleurine has been isolated from one species, another species has activity against L1210, and two species have high T/Cs in P388.

Subclass Caryophyllidae.—Only one active alkaloid, pilocereine, has been isolated from a cactus species (*Lophocereus schottii* [Engelm.] N. L. Britt. & Rose). Hence, this subclass is eliminated from further discussion.

Subclass Dilleniidae.—This subclass constitutes a fairly large element of the dicots (fig 6). Intensive screening has not uncovered any promising compounds (fig 20), although the activity data suggest that a closer examination of the Theales, Malvales, Violales, and Ebenales may be worthwhile.

Subclass Rosidae.—This subclass is the largest in the angiosperms and the richest in high-interest compounds and activity. Of the 16 orders in figure

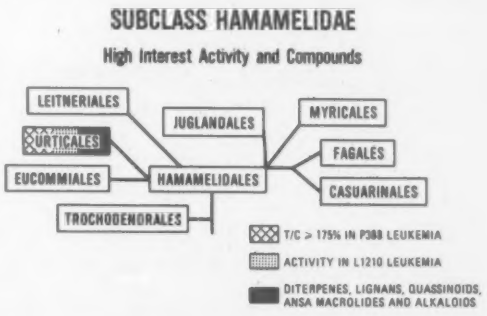

FIGURE 19.—Distribution of high-interest activity and compounds among the orders of Hamamelidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

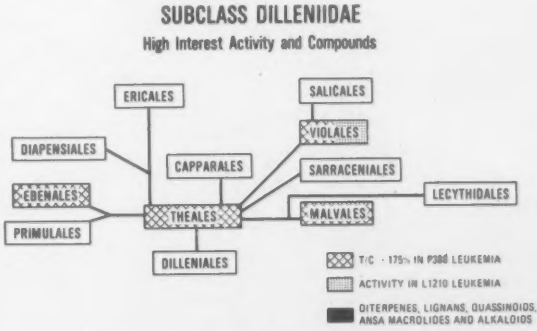

FIGURE 20.—Distribution of high-interest activity and compounds among the orders of Dilleniidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

TABLE 5.—HIGH-INTEREST COMPOUNDS IN ORDERS OF MAGNOLIIDAE

	Chemical Classes				
Orders	Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Magnoliales		+			+
Aristolochiales		+			
Ranunculales		+			+
Papaverales					+

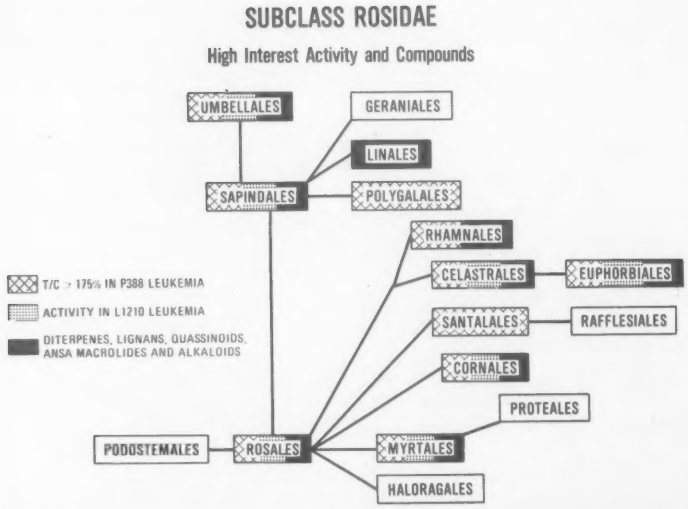

FIGURE 21.—Distribution of high-interest activity and compounds among the orders of Rosidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

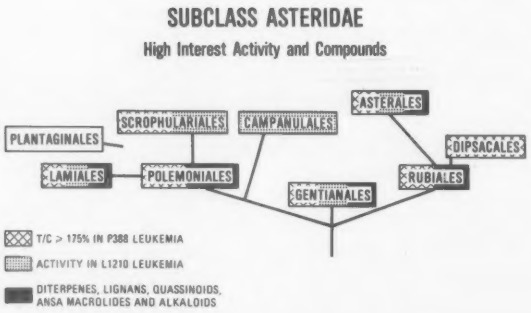

FIGURE 22.—Distribution of high-interest activity and compounds among the orders of Asteridae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

21, nine have high-interest compounds, seven have L1210 activity, and ten have high T/Cs in P388. Active compounds belonging to all five of the important chemical classes have been isolated from members of the Rosidae (table 6). These include seven of the 21 plant-derived compounds of greatest interest (1): the diterpenes—tripdiolide and triptolide; the quassinoid—bruceantin; the ansa macrolide—maytansine; and the alkaloids—camptothecin, fagaronine, and nitidine. This subclass also includes two compounds not belonging to the five important chemical classes but selected for preclinical pharmacology and possible clinical trial: Acer saponin P and the protein, cesalin. Although these compounds are inactive in the leukemias and slow-growing tumors (B16 and LL), they represent the best of two large chemical classes.

Some interesting chemotaxonomic relationships are revealed by an examination of table 6, where the sequence of orders follows Cronquist's classification (7), and figure 21, showing the probable relationships among the orders of the subclass.

Diterpenes.—Active diterpenes were isolated from plants in the orders Myrtales, Celastrales, and Euphorbiales.

Lignans.—Lignans are concentrated in three related orders: the Sapindales, Umbellales, and Linales.

Quassinoids.—This unique class of compounds is found in one family of the Sapindales.

Ansa macrolides.—These compounds were isolated from two species in closely related orders with a common origin: the Celastrales and Rhamnales. Ansa compounds were originally found as microbial products, and their occurrence in higher plants was once suspected to be due to microorganisms associated with the plant sources. However, the close relationship of the orders suggests that ansa macrolides may also be bona fide higher-plant products.

Alkaloids.—With the exception of the Euphorbiales, all of the orders containing alkaloids stem directly from the Rosales. Among the alkaloids isolated is camptothecin which occurs in both the Cornales and Celastrales.

Present knowledge indicates that members of the

TABLE 6.—HIGH-INTEREST COMPOUNDS IN ORDERS OF ROSIDAE

Orders	Chemical Classes				
	Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Rosales					+
Myrtales	+				+
Cornales					+
Celastrales	+			+	+
Euphorbiales	+				+
Rhamnales				+	
Sapindales		+	+		+
Linales		+			
Umbellales		+			

TABLE 7.—HIGH-INTEREST COMPOUNDS IN ORDERS OF ASTERIDAE

Orders	Chemical Classes				
	Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Gentianales					+
Polemoniales					+
Lamiales					+
Rubiales					+
Asterales					+

subclass Rosidae will play a major role in the future search for plant sources of anticancer drugs.

The Myrtales have not yet produced a compound destined for preclinical evaluation. However, on the basis of the diterpenes isolated from one family, the Thymelaeaceae, it is likely that this order will yield one or more such compounds. And, from data to be discussed later, the Euphorbiales are beginning to show real potential.

The Rosidae also appear promising from the activity data: 15 species in 13 genera and 11 families are active against L1210, and 95 species in 56 genera and 16 families have high T/Cs in P388. The T/C data identify two additional orders that deserve closer inspection: the Santalales and the Polygalales.

Subclass Asteridae.—This subclass, derived from the Rosidae, also has high potential. The only drugs from higher plants fully established in the treatment of cancer come from a member of this subclass: the vinca alkaloids from *Catharanthus roseus* (L.) G. Don in the family Apocynaceae of the order Gentianales. The occurrence of high-interest compounds and activity in the orders of subclass Asteridae is reflected in figure 22. In contrast to the

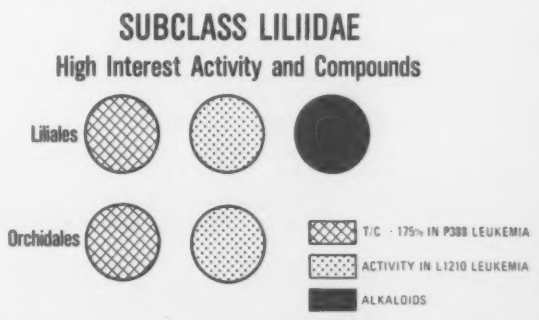

FIGURE 23.—Distribution of high-interest activity and compounds among the orders of Liliidae.

Rosidae, all of the high-interest compounds are alkaloids (table 7), including ellipticine, 9-methoxyellipticine, emetine, indicine-*N*-oxide, and tylocrebrine. This subclass also includes a compound on Hartwell's list not belonging to the five chemical classes: the quinone lapachol.

The activity data for the Asteridae are also highly promising: six species in six genera and five families

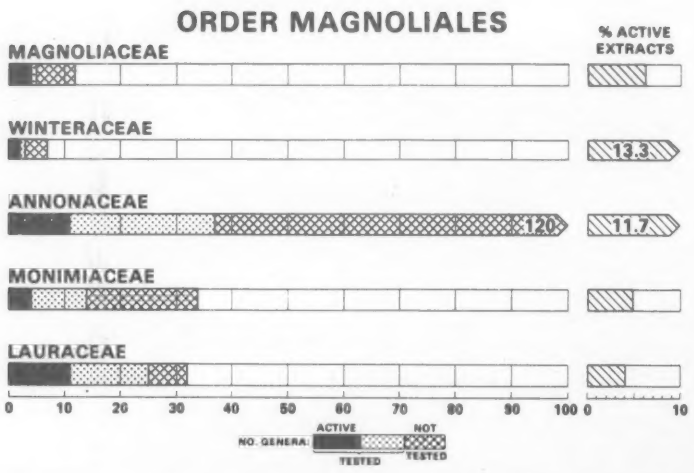

FIGURE 24.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 5 families of Magnoliales.

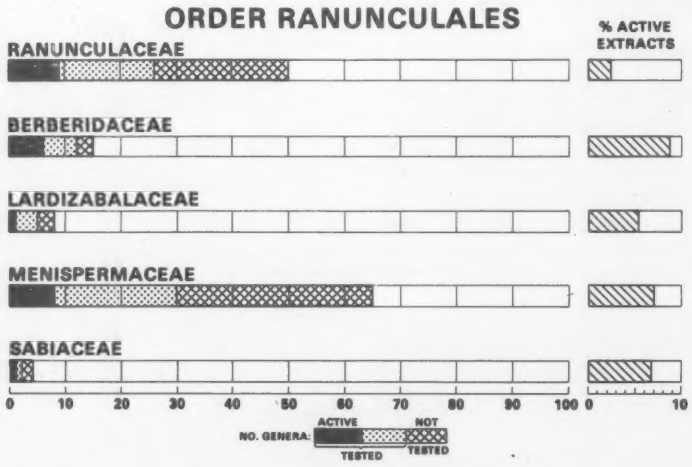

FIGURE 25.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 5 families of Ranunculales.

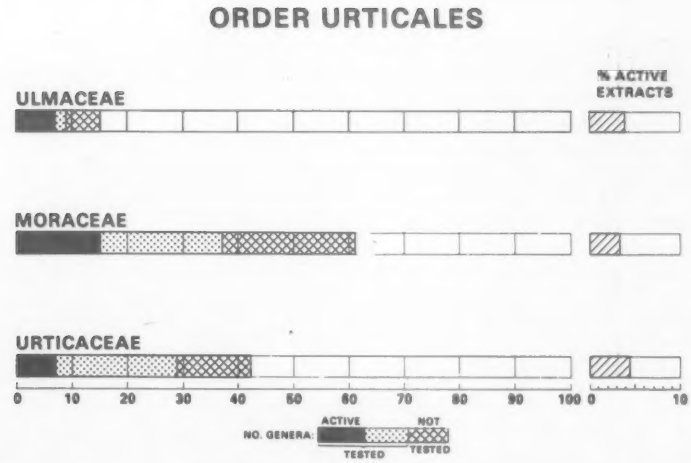

FIGURE 26.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the families of Urticales.

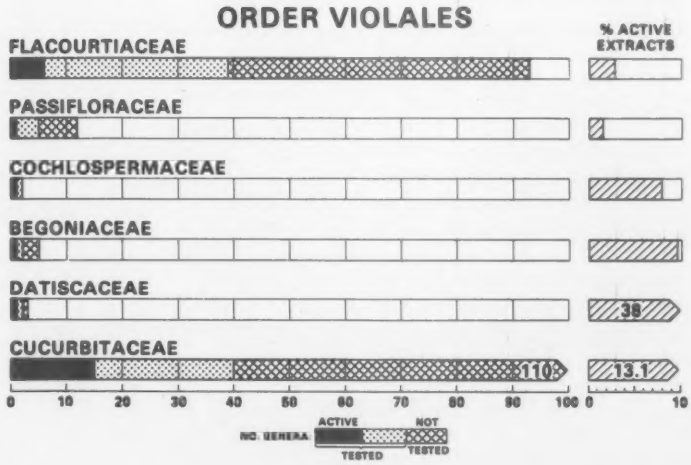

FIGURE 27.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Violales.

have activity against L1210 and 40 species in 34 genera and 12 families have high T/Cs in P388. The T/C data also indicate two orders which may merit special attention: the Scrophulariales and the Dipsacales.

The Asterales consist of a single family, the Asteraceae. About 12% of all species and 30% of all genera under consideration belong to this family. Yet, only nine species have high T/Cs in P388 and only one species has L1210 activity. Most of the activity in the Asteraceae is due to sesquiterpene lactones and other low-priority compounds. This family qualifies as a FONI, even though two active alkaloids have been isolated from a member of the genus *Senecio*.

Class Liliatae

Subclass Liliidae.—The only real potential in the monocots or Liliatae resides in this subclass which contains two orders: the Liliales and the Orchidales. As shown in figure 23, the high-interest compounds isolated from members of the Liliales are alkaloids, one of which is 3-desmethylcolchicine. Also in the Liliales, three species in two genera and two families have L1210 activity, and nine species in seven genera and four families have high T/Cs in P388.

Distribution of High-Interest Activity and Compounds in the Orders and Families of the Angiospermae[6]

By applying high-interest activity and compound criteria it was possible, in the preceding section, to narrow the field of subclasses and orders to those having the greatest potential as sources of anticancer agents (table 8). The same criteria will be applied to identify the most promising families in these groups.

Order Magnoliales (5600 species in 243 genera and 19 families).—Screened: 438/107/16. Active: 74/39/10. The three families not screened contain only one or two species each. Figure 24 illustrates the number of active, tested, and total genera and the percentage of active extracts in five of the most promising families. The yield of active extracts in the Winteraceae and Annonaceae is well above the 4% yield for the higher plants as a whole. Activity against

TABLE 8.—HIGH-INTEREST ACTIVITY AND COMPOUNDS IN ORDERS OF ANGIOSPERMAE

CLASS, SUBCLASS, AND ORDER	T/C in P388 = 175%	L1210 Activity	HIGH INTEREST COMPOUNDS*
Class Magnoliatae			
Subclass Magnoliidae			
Order Magnoliales	+		+
Order Aristolochiales	+		+
Order Ranunculales	+	+	+
Order Papaverales	+		+
Subclass Hamamelidae			
Order Urticales	+	+	+
Subclass Dilleniidae			
Order Theales	+		
Order Malvales	+		
Order Violales	+	+	
Order Ebenales	+		
Subclass Rosidae			
Order Rosales	+	+	+
Order Myrtales	+	+	+
Order Cornales	+	+	+
Order Santalales	+		
Order Celastrales	+	+	+
Order Euphorbiales	+	+	+
Order Rhamnales	+		+
Order Sapindales	+	+	+
Order Linales			+
Order Polygalales	+		
Order Umbellales	+	+	+
Subclass Asteridae			
Order Gentianales	+	+	+
Order Polemoniales	+		+
Order Lamiales	+	+	+
Order Scrophulariales	+	+	
Order Rubiales	+		+
Order Dipsacales	+		
Order Asterales	+	+	+
Class Liliatae			
Subclass Liliidae			
Order Liliales	+	+	+
Order Orchidales	+	+	•

*Presence of diterpenes, lignans, quassinoids, ansa macrolides or alkaloids

[6]Chemists under contract with the DR&DP, NCI, are working to isolate and characterize the active agents from many angiosperms with promising anticancer activity. To protect their interests in rights of discovery and publication, such plants are referred to by generic name only. As the active agents become known, they and their source plants are reported in the chemical literature.

both KB and P388 is well represented in the active families of this order, and one species of the Monimiaceae, *Siparuna* sp., has a T/C in P388 > 200%. Active alkaloids have been isolated from four species in four families: (-)-dicentrine from *Magnolia virginiana* L. of the Magnoliaceae, liriodendine from *Annona glabra* L. of the Annonaceae, cryptowolline iodide from *Cryptocarya laevigata* var. *bowiei* (Hook.) Kosterm. of the Lauraceae, and thalicarpine from *Hernandia ovigera* L. of the Hernandiaceae. Also, the active lignan, deoxypodophyllotoxin, has been isolated from *H. ovigera*. The overall screening results encourage continued sampling in this order.

Order Aristolochiales (600 species in seven genera and one family).—Screened: 40/5/1. Active: 8/4/1. Both high-interest compounds and activity occur in this family: *Asarum* sp. and *Aristolochia* sp. have high T/Cs in P388, and an active lignan, α-peltatin, has been isolated from *Asarum canadense* L.

Order Ranunculales (3200 species in 145 genera and eight families).—Screened: 321/78/8. Active: 42/26/6. The five most important active families are depicted in figure 25. The Ranunculaceae, Berberidaceae, and Menispermaceae have an excellent representation of high-interest types of compounds (table 9), one of which is the alkaloid thalicarpine. In addition, four species of the Berberidaceae have high-interest activity: two *Berberis* spp. with T/Cs in P388 of 200% or more, and sp. *Diphylleia* and *Podophyllum* sp. with activity against L1210. Although the Sabiaceae are, as yet, relatively unexplored, 100% of the extracts tested are active in P388, and one species of *Meliosma* has a T/C in P388 of ≥ 200%. The screening results indicate that at least three families of the Ranunculales are actual or potential FOSIs: the Ranunculaceae, Berberidaceae, and Menispermaceae.

Order Papaverales (600 species in 47 genera and

TABLE 9.—HIGH-INTEREST COMPOUNDS IN RANUNCULALES

COMPOUND	GENUS AND SPECIES	FAMILY
Alkaloids		
Berberine sulfate	Coptis teeta Wall.	Ranunculaceae
	Hydrastis canadensis L.	"
	Thalictrum rugosum Ait.	"
	Berberis asiatica Roxb. ex DC.	Berberidaceae
Cocsulinine	Cocculus pendulus (Forst.) Diels	Menispermaceae
Cycleadrine	Cyclea peltata Hook. fil. & Thoms.	"
Cycleanorine	" " " " " "	"
Cycleapeltatin	" " " " " "	"
Isochondrodendrine	" " " " " "	"
Obamegin	Xanthorrhiza simplicissima Marsh.	Ranunculaceae
Oxyacanthine	" " "	"
	Berberis asiatica	Berberidaceae
epi-Stephanine	Stephania hernandifolia (Willd.) Walp.	Menispermaceae
Stephavanine	S. abyssinica (Dillon & A. Rich.) Walp.	"
Thalicarpine	Thalictrum dasycarpum Fisch., C.A. Mey. & Ave.-Lall.	Ranunculaceae
Thalidasine	" " " " " " "	"
Lignans		
α-Peltatin	Diphylleia cymosa Michx.	Berberidaceae
β-Peltatin	" " "	"
Deoxypodophyllotoxin	Podophyllum pleianthum Hance	"
Podophyllotoxin	" " "	"

one family).—Screened: 58/19/1. Active: 10/6/1. Both high-interest activity and active compounds occur in the Papaveraceae (including the Fumariaceae). Four active alkaloids have been isolated from two species: berberine sulfate from *Argemone mexicana* L., and chelidimerine, coptisine chloride, and sanguidimerine from *Chelidonium majus* L. Moreover, one species of *Hunnemannia* has a T/C in P388 of ≥ 200%. Screening should continue; this family is known to be rich in alkaloids.

Order Urticales (2400 species in 118 genera and three families).—Screened: 141/75/3. Active: 58/29/3. Of the nine orders comprising the subclass Hamamelidae (fig 19), only the Urticales show any real potential; of the three families in this order (fig 26), only the Urticaceae or nettle family have both high-interest activity and compounds. The alkaloid cryptopleurine was isolated from *Boehmeria cylindrica* (L.) Sw., L1210 activity occurs in *Cypholophus* sp., and *Urera* sp. has a high T/C in P388. Aside from the Urticaceae, one species of *Ficus* in the Moraceae has a high T/C. Although activity is not spectacular in the order as a whole, it should be screened further.

Order Theales (3000 species in 155 genera and 15 families).—Screened: 257/66/9. Active: 28/16/5. Among the families comprising the order, high-interest activity occurs only in one species of *Calophyllum* and one of *Psorospermum* in the family Clusiaceae; both have T/Cs in P388 of ≥ 200%.

Order Malvales (3500 species in 239 genera and six families).—Screened: 519/123/5. Active: 40/30/5. Here, high-interest activity occurs in two families; *Sloanea* sp. in the Elaeocarpaceae and *Theobroma* sp. in the Sterculiaceae have high T/Cs in P388.

Order Violales (5200 species in 304 genera and 22 families).—Screened: 436/125/17. Active: 55/31/10. Six of the active families are shown in figure 27. The exceptionally high percentage of active extracts in the last three families—the Begoniaceae, Datiscaceae, and Cucurbitaceae—is now known to be due to the cucurbitacins, a group of highly toxic triterpenes of little interest to this program. In Cronquist's system (7), these families follow one another in a sequential numerical order indicating close relationship. All activity in this order is not necessarily due to cucurbitacins; since high T/Cs in P388 occur in two species of Flacourtiaceae (*Xylosma* sp. and *Zuelania* sp.), one species of Passifloraceae (*Passiflora* sp.), and one species of Cochlospermaceae (*Cochlospermum* sp.).

Order Ebenales (1700 species in 70 genera and six families).—Screened: 239/35/4. Active: 24/13/4. Although this order has yet to yield any high-interest compounds, species with high T/Cs in P388 occur in two families, *Manilkara* sp. in the Sapotaceae and *Styrax* sp. in the Styracaceae.

Order Rosales (20,000 species in 882 genera and 16 families).—Screened: 2576/457/11. Active: 256/124/6. The six active families comprise figure 28. With 12,000 species in 600 genera, the Fabaceae or legumes far outweigh the other families of this order in size. Of the 316 genera tested, 85 are active. Most of the activity, however, is due to tannins, sterols, quinones, rotenoids, and proteins (including cesalin from *Caesalpinia gilliesii* [Hook.] D. Dietr.). An active alkaloid, monocrotaline, was isolated from *Cro-*

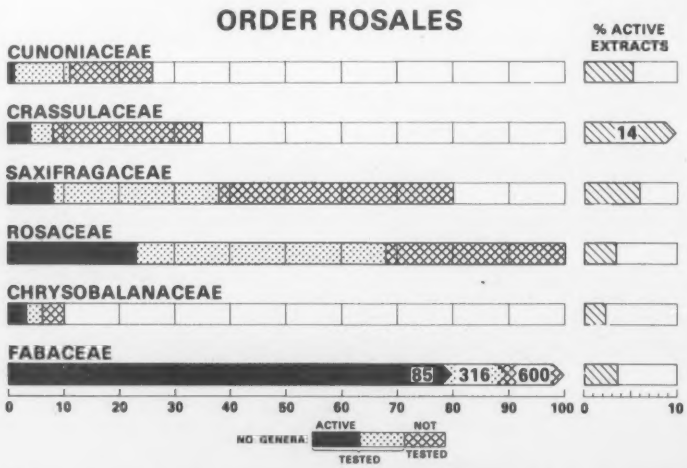

FIGURE 28.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Rosales.

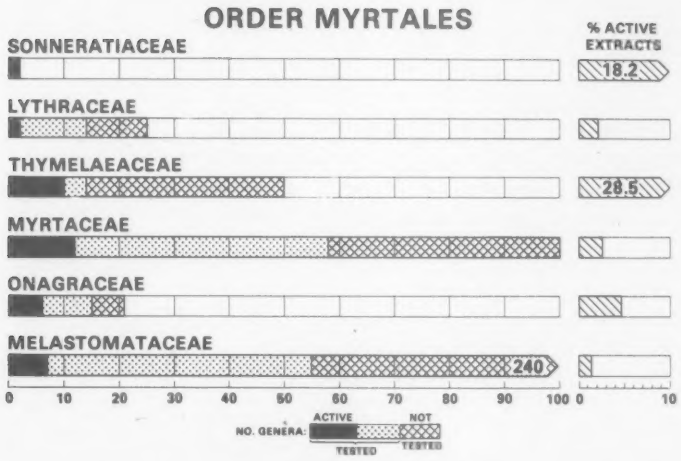

FIGURE 29.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Myrtales.

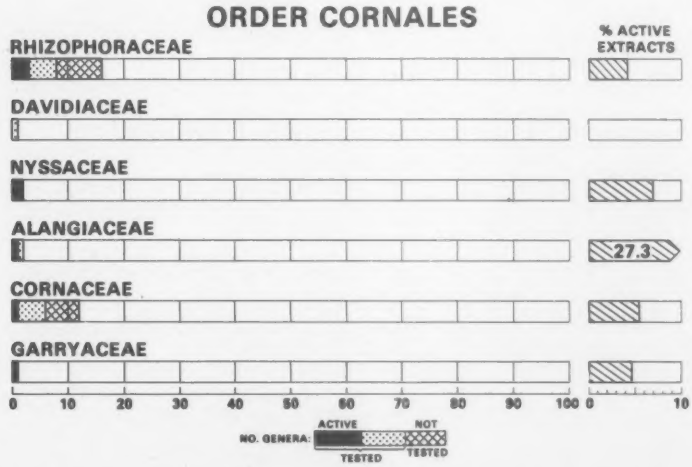

FIGURE 30.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in families of Cornales.

talaria spectabilis Roth. No extracts were active against L1210, and many of the extracts tested were active only against the obsolete tumor systems. Nevertheless, species in two closely related genera have T/Cs in P388 of ≥ 200%: two *Sesbania* spp. and *Glottidium* sp. Also, four legume species have high T/Cs in P388: *Astragalus* sp., *Calliandra* sp., *Cytisus* sp., and *Diphysa* sp. These may prove to be exceptions to the rule for the family.

The family Crassulaceae has a high number of active extracts and L1210 activity in one species of *Kalanchoe*. In the Rosaceae, *Horkelia* sp. and *Prunus* sp. have high T/Cs in P388, and *Vauquelinia* sp. has a T/C of ≥ 200%. The Chrysobalanaceae also have one species of *Parinari* with a T/C of ≥ 200%. Furthermore, active alkaloids have been isolated from two members of the Saxifragaceae: anopterine from *Anopterus macleayanus* F. Muell. and an unnamed alkaloid from *Dichroa febrifuga* Lour. All of these families need further exploration.

Order Myrtales (9000 species in 469 genera and 13 families).—Screened: 909/177/11. Active: 98/45/8. Figure 29 includes six of the active families. The unusual feature of this order is that the family Thymelaeaceae, a superb FOSI, is surrounded by a group of apparent FONIs. The only high-interest activity

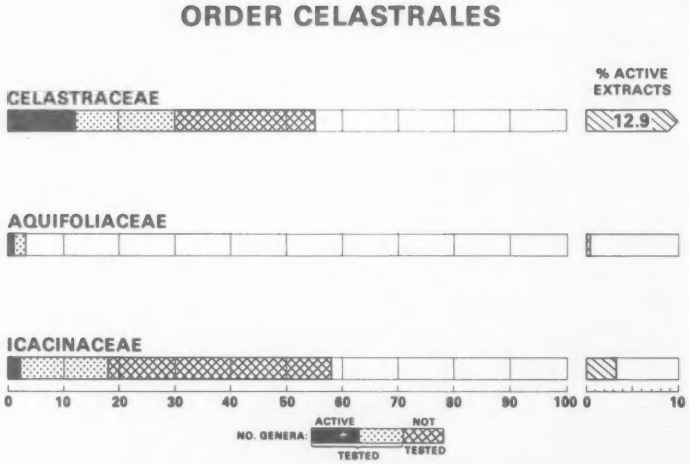

FIGURE 31.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 3 families of Celastrales.

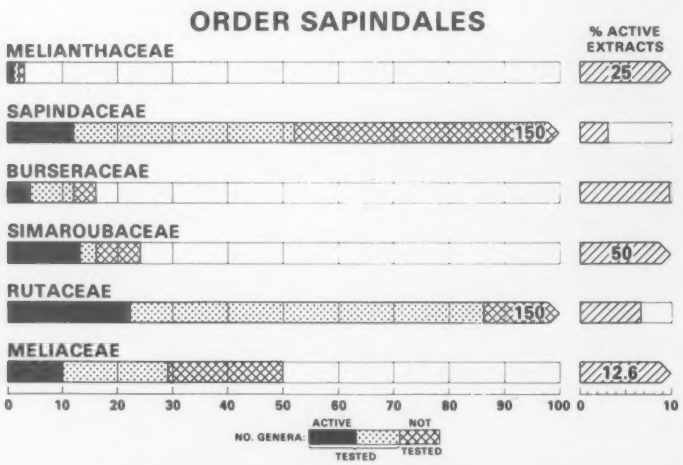

FIGURE 32.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Sapindales.

in the Myrtales is found in this family: *Daphne* sp. has L1210 activity and 14 species in six genera have high T/Cs in P388: five *Gnidia* spp., three *Wikstroemia* spp., two *Daphne* spp., two *Daphnopsis* spp., *Dais* sp., and *Pimelia* sp. In addition, potentially valuable diterpenes have been isolated from two species: 12-hydroxydaphnetoxin and mezerein from *Daphne mezereum* L., and gnidicin, gnididin, and gniditrin from *Gnidia laprantha* Gilg. The future prospects for the Thymelaeaceae are excellent. The very small family Sonneratiaceae has an excellent percentage of active extracts but, as yet, nothing is known of the chemistry.

Order Cornales (250 species in 34 genera and six families).—Screened: 74/19/6. Active: 15/8/5. All six families in this order are shown in figure 30. The Cornales produced camptothecin, one of the first compounds from higher plants to undergo clinical trial. This alkaloid comes from *Camptotheca acuminata* Decne. (Nyssaceae). The discovery of camptothecin prompted screening in closely related families, which led to the discovery of L1210 activity in

Alangium salviifolium (L. fil.) Wanger. and its active alkaloid, tubulosine. With the exception of the Alangiaceae, little promising activity has been found in the related families.

Order Santalales (2200 species in 134 genera and ten families).—Screened: 187/62/7. Active: 14/9/4. Although this order has not yet produced any high-interest compounds, all of the activity is against P388 and KB and two species have high T/Cs in P388: *Phoradendron* sp. of the Loranthaceae and *Schoepfia* sp. of the Olacaceae. Further screening may be profitable here.

Order Celastrales (2300 species in 128 genera and eight families).—Screened: 216/58/7. Active: 40/15/3. The three active families are represented in figure 31. Without a doubt, the Celastraceae (including the Hippocrateaceae) are one of the super FOSIs. The high-interest compounds isolated from members of this family (table 10) include three of the 21 most interesting compounds (1): tripdiolide and triptolide, diterpenes from *Tripterygium wilfordii* Hook. fil., and maytansine, an ansa macrolide from several species of *Maytenus*. In addition, 16 species in seven genera have high T/Cs in P388: nine *Maytenus* spp., two *Putterlickia* spp., *Euonymus* sp., *Hippocratea* sp., *Salacia* sp., *Wimmeria* sp., and *Tripterygium* sp.

A related family, the Icacinaceae, is also proving to be a promising source of high-interest compounds and activity. The species *Nothapodytes foetida* (Wight) Sleumer, an L1210 active, is a new source of camptothecin, as well as a derivative, 9-methoxy-camptothecin, not found in *Camptotheca*. In addition, a species of *Apodytes* has a T/C in P388 of 200% or more. Although the percentage of active extracts in this family has not been high, there is sufficient reason to screen further in the Icacinaceae.

By association, one might expect the Aquifoliaceae to be a promising source of activity, but this family, though widely screened, has produced little or nothing of interest.

Order Euphorbiales (8000 species in 301 genera and five families).—Screened: 674/128/5. Active: 89/33/2. There is good reason to believe that the Euphorbiales, an order derived from the Celastrales, may also prove to be a promising source of anticancer agents. This is especially true of the family Euphorbiaceae which, with 7500 species in 290 gen-

TABLE 10.—HIGH-INTEREST COMPOUNDS IN CELASTRACEAE

	GENUS AND SPECIES						
COMPOUND	*Maytenus buchananii* (Loes.) Wilczek	*Maytenus heterophylla* (Eckl. & Zeyh.) N. Robs.	*Maytenus serrata* (Hochst. ex A. Rich.) Wilczek	*Maytenus wightiana* Babu	*Putterlickia pyracantha* (L.) Endl.	*Putterlickia verrucosa* (E. Mey. ex Sond.) Szyszyl.	*Tripterygium wilfordii* Hook. fil.
Ansa macrolides							
Maysenine	+						
Maysine	+						
Maytanacine	+					+	
Maytanbutine	+	+	+	+		+	
Maytanprine	+	+	+	+	+	+	
Maytansine	+	+	+	+	+	+	
Maytansinol	+						
Maytanvaline	+			+			
Diterpenes							
Tripdiolide							+
Triptolide							+
Triptonide							+

TABLE 11.—HIGH-INTEREST COMPOUNDS IN SIMAROUBACEAE

COMPOUND	GENUS AND SPECIES: *Brucea antidysenterica* J.F. Mill.	*Brucea guineensis* G. Don	*Holacantha emoryi* Gray	*Picrasma excelsa* (Sw.) Planch.	*Pierreodendron kerstingii* Little	*Simarouba glauca* DC.
Quassinoids						
2'-Acetylglaucarubinone				+	+	
Ailanthinone					+	
Bruceantarin	+	+				
Bruceantin	+	+				
Bruceantinol	+	+				
Bruceine B	+	+				
Dehydroailanthinone					+	
Dehydrobruceantarin	+					
Dehydrobruceantin	+	+				
Dehydrobruceantol	+					
Glaucarubinone				+	+	+
Glaucarubolone					+	+
Holacanthone			+			
Lignans						
Dehydroanhydropicropodophyllin	+	+				

era, dwarfs the other families of the order. Of the two active families in this order, the Buxaceae and Euphorbiaceae, only the latter has high-interest activity: a species of *Garcia* is active against L1210 and 22 species in 13 genera have high T/Cs in P388: seven *Euphorbia* spp., two *Croton* spp., two *Jatropha* spp., two *Pycnocoma* spp., *Codiaeum* sp., *Garcia* sp., *Mallotus* sp., *Maprounea* sp., *Pausandra* sp., *Pedilanthus* sp., *Phyllanthus* sp., *Sapium* sp., and *Vernicia* sp. In addition, high-interest compounds have been isolated from two species: the diterpene, jatrophone, from *Jatropha macrorhiza* Benth., and the lignan, justicidin B, from *Phyllanthus brasiliensis* (Aubl.) Poir. The family Euphorbiaceae, also known to be rich in alkaloids, is definitely worthy of close attention. The other active family in the Euphorbiales, the Buxaceae, has yielded the active alkaloid, cycloprotobuxine, from *Buxus sempervirens* L.

Order Rhamnales (1670 species in 71 genera and three families).—Screened: 245/38/3. Active: 18/10/2. In this order, representatives of the Rhamnaceae and Vitaceae were active. However, high-interest activity and compounds occurred only in the family

TABLE 12.—HIGH-INTEREST COMPOUNDS IN RUTACEAE

COMPOUND	GENUS AND SPECIES
Berberine sulfate	*Zanthoxylum monophyllum* (Lam.) P. Wilson
Fagarone	*Fagara zanthoxyloides* Lam.
Lunasine chloride	*Lunasia amara* Blanco
	L. quercifolia (Warb.) Lauterb. & K. Schum.
5-Methoxycanthin-6-one	*Pentaceras australis* (F. Muell.) Hook. fil. ex Benth.
Methoxydihydronitidine	*Fagara leprieurii* (Guillem., Perr. & A. Rich.) Engl.
	F. macrophylla (Oliv.) Engl.
O-Methylfagaronine	*F. zanthoxyloides*
4-Methylthiocanthin-6-one	*Pentaceras australis*
Nitidine chloride	*Fagara chalybea* (Engl.) Engl.
	F. leprieurii
	F. macrophylla
	F. rubescens (Planch. ex Hook.) Engl.
	F. usambariensis Engl.
	Toddalia asiatica Lam.
	Zanthoxylum monophyllum
Oxynitidine	*Fagara macrophylla*

Rhamnaceae. Three species of the genus *Colubrina* have high T/Cs in P388 and three ansa macrolides have been isolated from *C. texensis* (Torr. & Gray) Gray: colubrinol, colubrinol acetate, and maytanbutine. It is interesting that maytanbutine has also been isolated from four species of the genus *Maytenus* (Celastraceae) of the order Celastrales.

The distribution of ansa macrolides may be of special phylogenetic significance. Among higher plants, ansa macrolides have appeared only in the Celastrales and Rhamnales, two orders which, according to Cronquist (7), have evolved from a common ancestor (*see* fig 21). The occurrence of ansa macrolides in only these orders corroborates Cronquist's hypothesis based on comparative morphology.

Because of the potential importance of ansa macrolides in anticancer research, the Rhamnaceae must be regarded as a FOSI. Furthermore, the closely related families Vitaceae and Leeaceae should receive special consideration.

Order Sapindales (6700 species in 517 genera and 16 families).—Screened: 903/265/14. Active: 155/80/11. Of the six active families (fig 32), two are definitely FOSIs (the Simaroubaceae and the Rutaceae), and at least two are very promising (the Burseraceae and the Meliaceae). The five active families not shown have neither high-interest compounds nor activity.

The small family Melianthaceae has excellent activity, but the active compounds isolated are primarily bufadienolides, which are of relatively low interest. On the more positive side, the active lignan, (+)-dimethylisolariciresinol-2 α-xyloside, has been isolated from *Bersama abyssinica* Fresen.

The least promising of the families (fig 32) appears to be the Sapindaceae because the activity is due to tannins.

In contrast to the Sapindaceae, the Burseraceae show considerable promise. A species of *Commiphora* has activity against L1210, and lignans have been isolated from three species of *Bursera:* deoxy-

podophyllotoxin, 5′-desmethoxy-β-peltatin A methyl ether, and β-peltatin A methyl ether from *B. fagaroides* (H.B.K.) Engl.; burseran and deoxypodophyllotoxin from *B. microphylla* Gray; and deoxypodophyllotoxin from *B. morelensis* Ramirez.

The active agents of the Simaroubaceae are the quassinoids, so far found only in this family. The high-interest compounds isolated from members of this family (table 11) include two of the 21 most interesting compounds (1): bruceantin from *Brucea antidysenterica* J. F. Mill. and *B. guineensis* G. Don and holacanthone from *Holacantha emoryi* Gray. Bruceantin is a strong candidate for preclinical toxicology. In addition, two species of *Brucea* are active against L1210 and 11 species in seven genera have high T/Cs in P388: three *Brucea* spp., two *Hannoa* spp., two *Simarouba* spp., *Picrasma* sp., *Picrolemma* sp., *Pierreodendron* sp., and *Quassia* sp.

The family Rutaceae with its promising alkaloids was early recognized as a FOSI. Nine active alkaloids have been isolated from 11 species in five genera (table 12). The active alkaloids include two strong candidates for preclinical toxicology (1): fagaronine and nitidine. In addition, two species of *Fagara* have activity against L1210 and the genera *Fagara* and *Zanthoxylum* each have three species with high T/Cs in P388.

The family Meliaceae merits special attention because not only does it have a good percentage of active extracts, but a species of *Guarea* has activity against L1210. Furthermore, three species in two genera have high T/Cs in P388: two *Aglaia* spp. and *Guarea* sp. With this percentage of active extracts and type of activity, the Meliaceae must be considered a FOSI, though nothing is known of the compounds responsible for the activity.

The family Aceraceae, not shown in figure 32, is noteworthy only because the previously mentioned Acer saponin P was isolated from *Acer negundo* L.

Order Linales (700 species in 29 genera and two families).—Screened: 45/9/2. Active: 10/2/2. Although the order has yet to produce high-interest activity, two active lignans have been isolated from *Linum album* Kotschy ex Boiss.: 3′-demethylpodophyllotoxin and podophyllotoxin.

Order Polygalales (1900 species in 87 genera and six families).—Screened: 122/41/6. Active: 14/11/6. So far, only tannins and phytosterols have been isolated from members of this order. However, one species of *Polygala* (family Polygalaceae) has a high T/C in P388.

Order Umbellales (3700 species in 330 genera and two families).—Screened: 332/129/2. Active: 22/19/2. The Araliaceae and Apiaceae, two closely related families, have promising species. In the Araliaceae, a species of *Schefflera* has activity against L1210 and a T/C in P388 of ≥ 200%, and a species of *Oplopanax* has a high T/C. In the Apiaceae, four active lignans have been isolated from *Steganotaenia araliacea* Hochst.: steganacin, steganangin, steganol, and steganone. Further screening in both of these families should be profitable.

Order Gentianales (5600 species in 408 genera and four families).—Screened: 600/171/4. Active: 117/52/3. The four families in this order (fig 33) run the gamut from super FOSI to complete FONI.

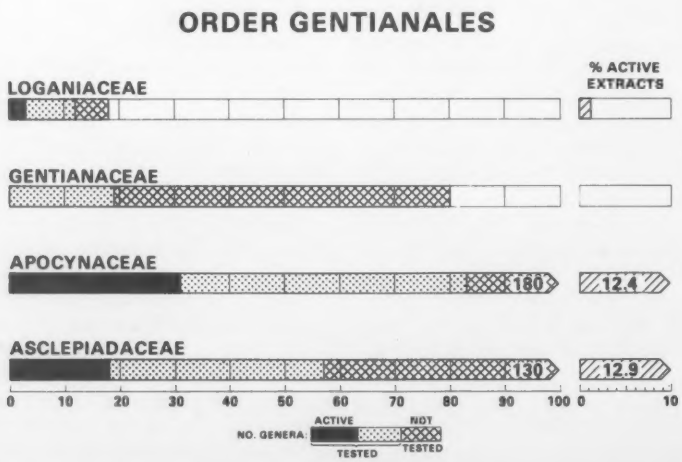

FIGURE 33.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the families of Gentianales.

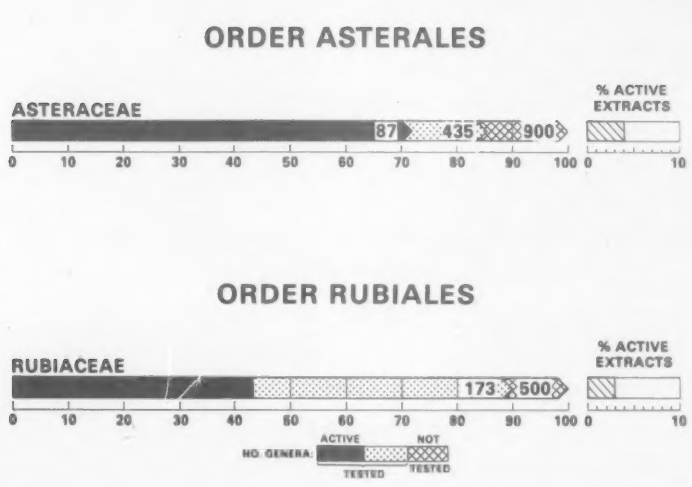

FIGURE 34.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the Rubiales and Asterales.

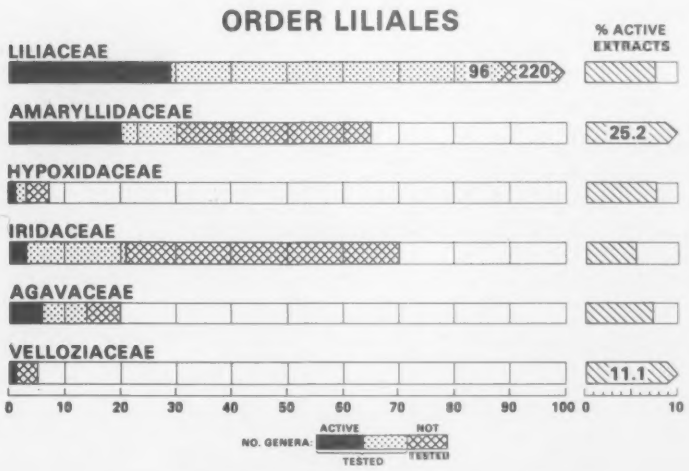

FIGURE 35.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Liliales.

The family Apocynaceae is the sole source of higher plant compounds used in the treatment of cancer; these are the vinca alkaloids from *Catharanthus roseus*. Furthermore, seven active alkaloids have been isolated from eight species in six genera (table 13).

Ellipticine and 9-methoxyellipticine are among the 21 most interesting compounds (1). In addition, ten species in six genera have high T/Cs in P388: five *Tabernaemontana* spp., *Alstonia* sp., *Catharanthus* sp., *Ervatamia* sp., *Schizozygia* sp., and *Stemmadenia* sp. Continued intensive screening is definitely indicated for the Apocynaceae.

The Asclepiadaceae, closely related to the Apocynaceae, must be regarded as a potential FOSI. As in the Apocynaceae, much of the activity here is due to cardenolides and the best activity is due to alkaloids. Five active alkaloids have been isolated from four species of the genus *Tylophora* (table 14), including one, tylocrebrine, on the list of the 21 most interesting compounds (1).

The family Gentianaceae is a classic example of a FONI. After screening 52 species in 19 genera, not a single active species has materialized. Although the Loganiaceae contain three active species, the family is lacking in high-interest activity or compounds and is only of marginal interest.

Order Polemoniales (4600 species in 187 genera

TABLE 13.—HIGH-INTEREST COMPOUNDS IN APOCYNACEAE

COMPOUND	GENUS AND SPECIES
Conessine hydrochloride	Holarrhena antidysenterica (L.) Wall. ex A. DC.
Ellipticine	Excavatia coccinea (Teysm. & Binnend.) Markgraf
	Ochrosia moorei (F. Muell.) F. Muell.
Leurosine	Catharanthus lanceus (Boj. ex A. DC.) Pinchon
	C. pusillus (Murr.) G. Don
9-Methoxyellipticine	Excavatia coccinea
	Ochrosia moorei
Reserpine	Excavatia coccinea
Voacamine	Tabernaemontana johnstonii (Stapf) Pinchon
	T. ventricosa Hochst. ex A. DC.
	Voacanga africana Stapf ex G. Ell.
Voacorine	V. africana

TABLE 14.—ACTIVE ALKALOIDS IN TYLOPHORA

COMPOUND	GENUS AND SPECIES
Desmethyltylophorinine	T. dalzellii Hook. fil.
	T. indica (Burm. fil.) Merr.
Oxytylocrebrine	T. crebriflora S. T. Blake
	T. hirsuta (Wall.) Wight
Tylocrebrine	T. crebriflora
	T. hirsuta
Tylophorine	T. crebriflora
	T. hirsuta
	T. indica
Tylophorinine	T. crebriflora
	T. indica

and seven families).—Screened: 575/103/7. Active: 63/27/4. In this order, high-interest compounds have been isolated only from members of the Solanaceae, and they too, are alkaloids: solaplumbin, solasodine base, solasodine hydrochloride, and solasodine rhamnoside (*Nicotiana plumbaginifolia* Viv.); β-solamarine (Solanum dulcamara L.); and solapalmatenine and solapalmatine (*Solanum tripartitum* Dun.). High-interest activity has been detected in only one species of the Convolvulaceae: *Argyreia* sp., with a high T/C in P388.

Order Lamiales (7800 species in 395 genera and five families).—Screened: 973/194/5. Active: 60/37/3. All three active families appear to be promising. The family Boraginaceae is the source of indicine-*N*-oxide, an alkaloid isolated from *Heliotropium indicum* L., and included among the 21 most interesting compounds (1). Among the species in the Verbenaceae, *Citharexylum* sp. is active against L1210 and *Cornutia* sp. has a high T/C in P388. Also, three species of the Lamiaceae have high T/Cs in P388: *Hyptis* sp., *Roylea* sp., and *Lepechinia* sp. Nothing is known of the compounds responsible for this activity in the Verbenaceae and Lamiaceae.

Order Scrophulariales (10,000 species in 795 genera and 13 families).—Screened: 924/268/12. Active: 60/37/6. In the context of this paper, no high-interest compounds have been isolated from members of this order. However, it should be noted that the quinone, lapachol, from *Stereospermum suaveolens* (Roxb.) DC. of the Bignoniaceae, is included on the list of the 21 most interesting compounds (1). High-interest activity is found in three families. *Digitalis* sp. of the Scrophulariaceae has activity against L1210, and three species have high T/Cs in P388: *Stereospermum* sp. of the Bignoniaceae, *Schrebera* sp. of the Oleaceae, and *Penstemon* sp. of the Scrophulariaceae.

Order Rubiales (6500 species in 500 genera and one family).—Screened: 591/173/1. Active: 62/43/1. The single family Rubiaceae (fig 34) is a source of both high-interest compounds and activity. The alkaloid, emetine, from *Cephaelis ipecacuanha* (Brot.) A. Rich. is among the 21 most interesting compounds (1). In addition, six species in six genera have high T/Cs in P388. The genera are *Bouvardia*, *Feretia*, *Genipa*, *Pogonopus*, *Psychotria*, and *Rubia*. This family is known to be rich in alkaloids and is definitely worthy of further investigation.

Order Dipsacales (1100 species in 44 genera and five families).—Screened: 152/27/4. Active: 15/5/2. Although the active families, Caprifoliaceae and Valerianaceae, have yet to produce a high-interest compound, both have representatives with high T/Cs in P388: *Lonicera* sp., *Sambucus* sp., and *Viburnum* sp. of the Caprifoliaceae and *Valeriana* sp. of the Valerianaceae.

Order Asterales (19,000 species in 900 genera and one family).—Screened: 2484/435/1. Active: 177/87/1. This order consists of the single family Asteraceae, one of the largest families of flowering plants. Considering the extent to which this family has been screened, the yield of high-interest compounds has been practically nil. So far, only the alkaloids senecionine and senecionine-*N*-oxide, from *Senecio triangularis* Hook., are high-interest types. The active compounds isolated are mainly sesquiterpene lactones and other low-interest compounds such as phytosterols and saponins. Also, the yield of high-interest activity has been poor: only a species of *Oligochaeta* has activity against L1210, and nine species in eight genera have high T/Cs in P388: two *Helenium* spp., *Baccharis* sp., *Carthamnus* sp., *Eriophyllum* sp., *Hymenoclea* sp., *Senecio* sp., *Xanthocephalum* sp., and *Zaluzania* sp. Based on past screening experience, it would be reasonable to expect that most of this activity is due to sesquiterpene lactones, and it is likely that the L1210 active will turn out to be a false-positive. The bar graphs in figure 34 provide a good comparison of the general activity in two very large families, the Rubiaceae and Asteraceae. Of these two families, the Asteraceae would appear to be the more promising source of anticancer agents but, as has been seen in similar cases, the activity in the Asteraceae is largely useless due to the nature of the responsible agents. And, for the same reason, the activity in the Rubiaceae is potentially valuable.

Order Liliales (7700 species in 445 genera and 14 families).—Screened: 666/176/13. Active: 117/54/6. Among the monocot orders, both high-interest compounds and activity occur only in the Liliales. Of the six active families depicted in figure 35, two are definitely FOSIs—the Liliaceae and Amaryllidaceae; the family Agavaceae is an apparent FONI.

The high-interest compounds in the Liliaceae are alkaloids: five have been isolated from *Colchicum speciosum* Stev. and one from *Urginea altissima* (L. f.) J. G. Baker (table 15). One of the alkaloids, 3-desmethylcolchicine, is included on the list of the 21 most interesting compounds (1). In addition, two *Colchicum* spp. have activity against L1210, and five species in three genera have high T/Cs in P388: two *Colchicum* spp., two *Merendera* spp., and *Urginea* sp.

The Amaryllidaceae are also a promising source of high-interest compounds and activity. Here again, the high-interest compounds are alkaloids: two have been isolated from *Crinum macrantherum* Engl. and one from *Hymenocallis latifolia* (Mill.) M. J. Roem. (table 15). A species of *Pancratium* has

TABLE 15.– HIGH-INTEREST COMPOUNDS IN LILIALES

COMPOUNDS	FAMILY, GENUS AND SPECIES			
	LILIACEAE		AMARYLLIDACEAE	
	Colchicum speciosum Stev.	*Urginea altissima* (L. fil.) J.G. Baker	*Crinum macrantherum* Engl.	*Hymenocallis latifolia* (Mill.) M.J. Roem.
Alkaloids:				
Colchicine	+			
Crinamine			+	
Demecolcine	+			
3-Desmethylcolchicine	+			
N-Formyldesacetylcolchicine	+			
Lycorine			+	+
Scilliglaucosidin		+		

activity against L1210 and two species have high T/Cs in P388: *Amaryllis* sp. and *Pancratium* sp. Also, the percentage of active extracts is exceptionally high in this family.

The family Agavaceae, thought to hold great promise early in the program, now appears to be a FONI. The activity is overwhelmingly due to saponins, but other unimportant compounds like tannins and cucurbitacins have also been isolated. However, one species of *Cordyline* has a high T/C in P388.

Little is known about the remaining families. However, there are promising indications: 100% of the extracts tested from members of Hypoxidaceae and Velloziaceae are active against P388, and one species of the Iridaceae (*Iris* sp.) has a high T/C in P388. All three families are worthy of further exploration.

COMMENTS AND CONCLUSIONS

In this symposium, Hartwell (1) demonstrated that a wide variety of chemical structures are active against tumors in laboratory animals. Under the present screening criteria, relatively few of these compounds appear to have potential as anticancer agents. Nevertheless, the activity against all tumor systems, current and obsolete, represents a wealth of information on biologic activity of higher plant products, information which transcends the immediate search for plant sources of anticancer drugs.

Thanks to the random nature of procurement and screening, the more than 20,000 species encompassed in this investigation represent a broad spectrum of the higher plants. Throughout this paper, the numbers or percentages of active, tested, and total genera illustrate the extent of screening and anticancer activity in the major divisions of the vascular plants, in the orders of the gymnosperms, and in the major orders comprising the ten subclasses of the angiosperms. These generic data, as well as the percentage yields of active extracts, are shown for selected families belonging to orders of the gymnosperms and angiosperms known to be sources of high-interest compounds and/or activity.

The distribution of the overall positive and negative screening results in taxonomic groups of higher plants shows their relative yields of biologic activity, but not necessarily their yields of useful anticancer activity or agents. Some of the reasons for the inadequacy of the general, broad-spectrum activity to identify useful anticancer agents are brought out.

The continuing search for plant sources of anticancer drugs should take into account the systematic distribution in plants of chemical compounds producing activity against those test systems most predictive for clinical efficacy. The three high-interest criteria selected for the present taxonomic analysis are: (*a*) the five most important classes of chemical compounds: diterpenes, lignans, quassinoids, ansa macrolides, and alkaloids; (*b*) activity against L1210; and (*c*) high T/Cs in P388. The screening criteria selected were shown in this study to emphasize those taxonomic groups already known to be important sources of compounds. The application of these criteria should indicate where to locate new sources of known high-interest compounds and

provide leads to sources of related or novel high-interest compounds.

The above criteria serve not only to narrow the field of higher plants to those taxonomic groups with the most potential, but also to identify those groups with little or no potential.

The high-interest compounds with the plant species from which they were isolated and the genera with high-interest activity are cited under the orders and families in which they occur.

Whether a given plant family is a FOSI or a FONI or lies somewhere between these extremes depends on the extent of screening, the yield of general activity, and the presence or absence of high-interest compounds and/or activity. Certain special cases of clear-cut FOSIs and FONIs are brought out. This same arbitrary ranking can apply to taxonomic groups above and below the family level. Taxonomists, however, for mnemonic and didactic reasons, tend to think in terms of the family unit. Hence, the FOSI/FONI concept evolved.

The new procedure involving preliminary fractionation to concentrate active agents before screening (4) may significantly alter the FOSI/FONI concept. It has already increased the yields of plants active against P388 and KB. It remains to be seen whether the new procedure will redefine those families now believed to be FONIs.

Other papers in this symposium (1,11) suggest that folk uses of plants may provide valid guidelines for selective procurement and screening. Although such guidelines could be established, limiting the search to these plants would certainly reduce the opportunities for chance discoveries offered by random screening.

In 1969, Perdue and Hartwell (3) concluded that "the search for plant sources of anticancer drugs must be a long-term program," and the results of the present investigation emphasize that the same holds true today. There are few shortcuts to the successful exploration, discovery, and evaluation of anticancer agents in higher plants. The strongest evidence supporting this is the fact that compounds of potential value in cancer chemotherapy are widely distributed among many groups of the higher plants.

REFERENCES

1. HARTWELL JL. Types of anticancer agents isolated from plants. Cancer Treat Rep 60:1031–1067, 1976.
2. PERDUE RE, JR. Procurement of plant materials for antitumor screening. Cancer Treat Rep 60:987–998, 1976.
3. PERDUE RE, JR, and HARTWELL JL. The search for plant sources of anticancer drugs. Morris Arboretum Bull 20:35–58, 1969.
4. STATZ D, and KOON FB. Preparation of plant extracts for anticancer screening. Cancer Treat Rep 60:999–1005, 1976.
5. HARTWELL JL, and ABBOTT BJ. Antineoplastic principles in plants: recent developments in the field. *In* Advances in Pharmacology and Chemotherapy (Garattini S, Goldin A, Hawking F, et al, eds). New York, Academic Press, 1969, vol 7, pp 117–209.
6. AIRY SHAW HK. J. C. Willis's Dictionary of Flowering Plants and Ferns, 8th ed. London, Cambridge University Press, 1973, 1245 pp.
7. CRONQUIST A. The Evolution and Classification of Flowering Plants. Boston, Houghton Mifflin Co, 1968, 396 pp.
8. MELCHIOR H, and WERDERMANN E, eds. A. Engler's Syllabus der Pflanzenfamilien, 12th ed. I. Band. Berlin, Gebruder Borntraeger, 1954, 367 pp.
9. MELCHIOR H, ed. A. Engler's Syllabus der Pflanzenfamilien, 12th ed. II. Band. Berlin, Gebruder Borntraeger, 1964, 666 pp.
10. ABBOTT BJ. Bioassay of plant extracts for anticancer activity. Cancer Treat Rep 60:1007–1010, 1976.
11. SPJUT RW, and PERDUE RE. Folklore: a tool for predicting sources of antitumor activity among higher plants? Cancer Treat Rep 60:979–985, 1976.

Magnification of Plant Parts:

Habit x ½
Flower x 5
Calyx x 5
Petal x 6 (outside and inside views)
Samara x 1
Seed x 5 (ventral, lateral, dorsal views)
Older panicle with fruit x ½

Tripterygium wilfordii Hook. fil.—The source of triptolide and tripdiolide, this plant is a woody vine-like rambling shrub native to China and the mountains at the northern tip of Taiwan. The active agents are concentrated in the roots. The plant is cultivated in China as the source of an insecticide. It is propagated easily from cuttings and can be cultivated in the United States.

Novel Plant-Derived Tumor Inhibitors and Their Mechanisms of Action[1,2]

S. Morris Kupchan[3,4,*]

SUMMARY

The activity-directed isolation of new tumor inhibitors of plant origin has yielded many novel compounds with significant growth-inhibitory properties. A large proportion of the new compounds contain highly electrophilic functionalities and chemical and biochemical studies are yielding a growing body of evidence to support the view that these compounds may act by selective alkylation of growth-regulatory biologic macromolecules. The selectivity may result from many factors, among which are transport of the tumor inhibitor into the cell and the chemical nature and steric environment of the specific nucleophile to be alkylated. Model studies support the hypothesis that the inhibition of tumor growth by the new agents may be attributable to selective alkylation of key enzymes which control cell division.

[Cancer Treat Rep 60:1115–1126, 1976]

Modern cancer chemotherapy is considered by many to have started with the discovery of the antileukemic activity of mechlorethamine in the 1940s (1). An enormous amount of work has followed on the synthesis and evaluation of biologic alkylating agents (2,3). Although the preparation of modifications of presently known alkylating agents continues, some pessimism is evident among investigators in the field. The extensive synthetic efforts to date have led to only minimal improvements over the prototype drugs. Evidently, the high degree of reactivity of simple alkylating agents leads to indiscriminate reactions with many cell constituents and, consequently, narrow therapeutic indexes. There is a need for new prototypes for the synthetic organic chemist to use in the design of selective alkylating agents.

Recent studies on the isolation and structural elucidation of plant-derived tumor inhibitors are yielding a fascinating array of novel types of growth-inhibitory compounds. An ever-increasing number of the new compounds possess structures and chemical properties which suggest that they may act by selective alkylation of growth-regulatory macromolecules. This approach may yield sorely needed templates for the synthesis of potentially superior chemotherapeutic agents. As a corollary statement, the new growth inhibitors may provide powerful tools for the elucidation of new biochemical mechanisms of growth control that may be more amenable to selective regulation.

Studies of plant-derived tumor inhibitors are proceeding in many laboratories of wide geographic distribution; however, I will review only pertinent studies from my laboratory.

The program started modestly in 1959 with a screening study of crude extracts of a limited number of accessible plants for inhibitory activity against animal tumor systems. Some plants were procured by summer collections in Wisconsin, others by cooperative arrangements with botanists in India, Costa Rica, and other countries.

The results of testing of the first plant extracts prepared in this laboratory and elsewhere revealed that a small but significant number of the extracts showed reproducible tumor-inhibitory activity. Encouraged by these results, the NCI arranged with

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[2]This is part 113 in the author's series entitled "Tumor Inhibitors."

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[4]I would like to thank my collaborators and students whose names are given in the various references for their skill and devotion; I cannot adequately express my indebtedness to them.

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the US Department of Agriculture to procure several thousand plant samples per year for evaluation. Shortly thereafter, the NCI arranged a contract with the Wisconsin Alumni Research Foundation (Madison) to execute the initial extraction and screening studies. From that point onward, our program concentrated on the isolation and structural elucidation of new tumor inhibitors. To date, the active principles of > 150 plants have been isolated in our program, and the chemical studies of some of the most interesting compounds constitute the focus of this review.

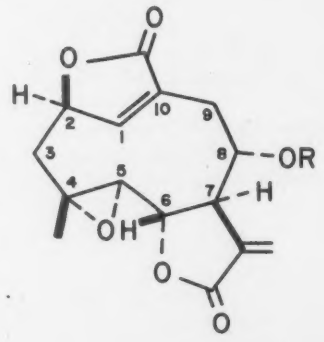
One aspect of the approach of our program differs significantly from the classic and most widely practiced approach to the biologic study of plant constituents. In the classic phytochemical approach, those compounds are studied which are most easily separated from a plant extract and most easily crystallized. In our program, however, the fractionation and isolation studies are guided at every stage by biologic assays; this has made possible the isolation of important minor constituents which might have been missed in the classic approach.

A consequence of our activity-directed fractionation-isolation procedure is the absence of any prejudicial selection of any particular class of compounds. Nevertheless, new and interesting relationships between chemical reactivity and growth-inhibitory activity are emerging. I will focus here on the noteworthy chemical similarity of many of the tumor inhibitors that we have isolated, namely, the presence of highly electrophilic functions. The structures and reactivities of these compounds support the hypothesis that their tumor-inhibitory activity may be associated with selective alkylation of nucleophilic groups in enzymes that control cell division.

CHEMISTRY OF THE NEW TUMOR INHIBITORS

A systematic study of the cytotoxic principles of *Elephantopus elatus* Bertol. led to the isolation of two novel tumor-inhibitory germacranolide dilactones, elephantin and elephantopin. Although the compounds were concentrated and isolated solely on the basis of in vitro cytotoxicity, elephantopin has subsequently shown significant inhibitory activity against the P388 lymphocytic leukemia in the mouse and the Walker 256 carcinosarcoma in the rat. (The KB cytotoxicity assay has been invaluable in many of our other isolation studies as well. Significant results were frequently obtained from the bioassay of 2–10-mg samples of materials which could be evaluated in animal tumor systems in vivo only at the cost of 0.5–1.0-g samples.) A combination of degradative, spectral, and X-ray crystallographic studies resulted in elucidation of the structures of elephantin and elephantopin (fig 1) (4,5).

Studies of *Vernonia hymenolepis* A. Rich led to the isolation and structural elucidation of two novel elemanolide dilactones, vernolepin and vernomenin. Vernolepin showed significant in vitro cytotoxicity and in vivo tumor-inhibitory activity against the Walker 256 carcinosarcoma in the rat. Elemental analysis and mass spectrometry indicated a $C_{15}H_{16}O_5$ molecular formula for vernolepin. Chemical and spectral evidence indicated the presence of two α, β-unsaturated lactone functions, a secondary alcohol, an additional double bond, and, therefore, a monocarbocyclic ring skeleton. The structure and stereochemistry for vernolepin, depicted in figure 2, were established by X-ray crystallographic analysis of the *p*-bromobenzenesulfonate. Vernomenin showed chemical properties similar to vernolepin, and its structure was proven by (*a*) its conversion to the same methanol adduct as that obtained from vernolepin, and by (*b*) a comparison of the NMR spectra of the respective acetate esters. In vernomenin acetate, the triplet centered at τ 4.78 could be assigned to the proton at acetate-bearing carbon 6, while the multiplet centered at τ 5.90 corresponded to the proton (spin-coupled to three protons) at lactone-oxygen-bearing carbon 8. In contrast, the spectrum of vernolepin acetate showed a multiplet at τ 4.95, assigned to the proton at acetate-bearing carbon 8, while the lactone proton signal appeared as a triplet centered at τ 5.96, indicative of attachment to carbon 6 (6,7).



Elephantin : R : $(CH_3)_2C=CHCO-$

Elephantopin : R : $CH_2=C(CH_3)CO-$

FIGURE 1.—Principles of *Elephantopus elatus* active against Walker 256 carcinosarcoma.

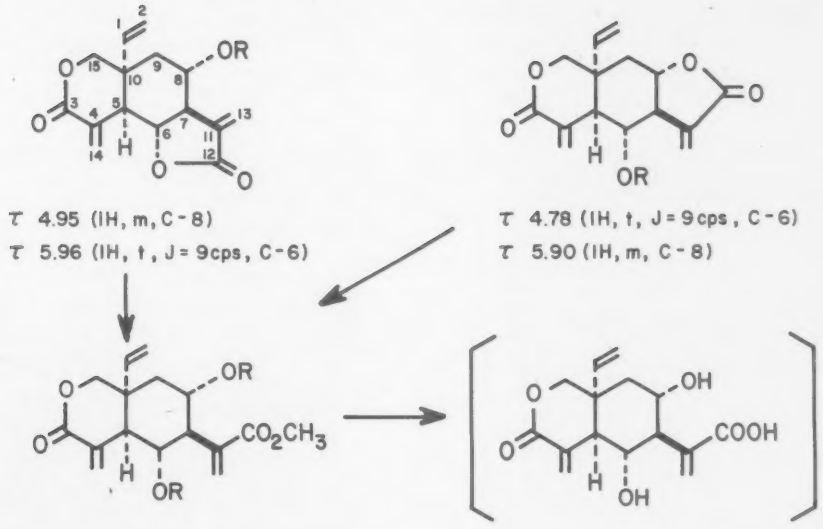


FIGURE 2.—Structures of vernolepin and vernomenin.

In an attempt to elucidate a possible function of a series of new plant-derived tumor inhibitors, we collaborated with Professor Luis Sequeira to evaluate the effects of these compounds on plant growth. Several of the sesquiterpene dilactones (specifically, elephantin, elephantopin, and vernolepin) were found to be strong inhibitors of the extension growth of wheat coleoptile sections (8). Vernolepin inhibits extension growth in concentrations of 5–50 μg/ml (from 20% to 80%, fig 3). If the inhibited sections are washed and subsequently treated with indole-3-acetic acid, the tissues respond to the auxin, but the degree of elongation is determined by the length of prior treatment with vernolepin. The fact that vernolepin's plant-growth-inhibitory activity is reversible suggests that the compound may have a natural function in the regulation of plant growth.

Several recent observations have focused attention on the importance of the conjugated α-methylene lactone function for the biologic activity of the sesquiterpene lactones. For instance, the plant-growth-inhibitory effect of vernolepin is completely blocked by addition of sulfhydryl compounds such as mercaptoethanol to the medium. Second, as shown in figure 4, the cytotoxicity of vernolepin derivatives appears to be directly related to the presence of free conjugated α-methylene lactone functions. Thus, selective reduction of the ethylidene double bond does not appear to affect the cytotoxicity. However, modification of the α-methylene-γ-lactone (by *trans*-esterification to the methanol adduct or by hydrogenation) results in a tenfold diminution in cytotoxicity. Modification of both α-methylene lactone systems, as in hexahydrovernolepin, leads to a derivative that is essentially inactive.

We have studied the reactions of several conjugated α-methylene lactones with model biologic nucleophiles. The rates of the reactions of vernolepin and elephantopin with cysteine at pH 7.4 were determined spectrophotometrically (fig 5). The most reactive function in each case was the conjugated α-methylene-γ-lactone, and the second-order rate con-

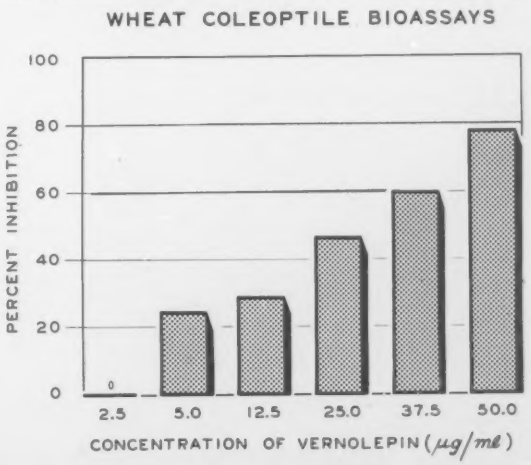


FIGURE 3.—Wheat coleoptile bioassays.

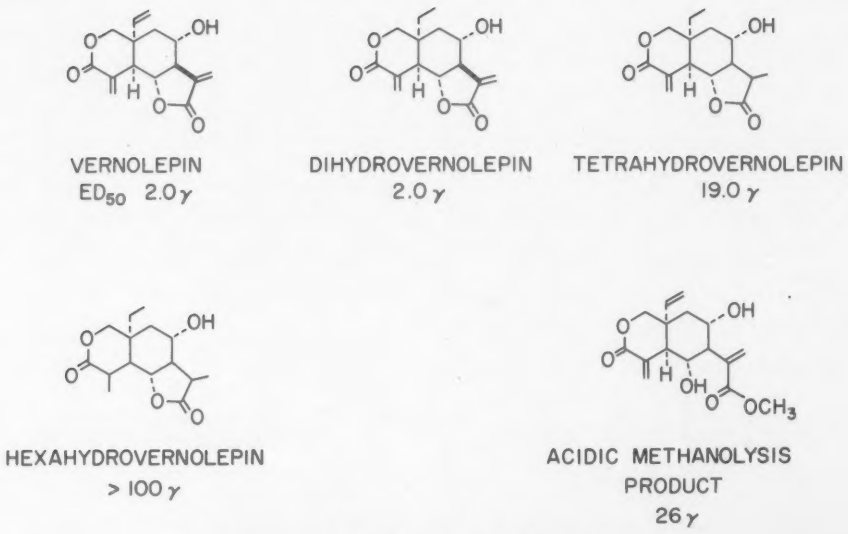


FIGURE 4.—Cytotoxicity of vernolepin derivatives.

stants for the Michael-type addition of cysteine showed the same order of reactivity toward cysteine as iodoacetate, a commonly used sulfhydryl reagent. In contrast to the reactivity of the lactones toward sulfhydryl groups, their reaction with amino groups appeared to be very slow. When a solution equimolar in lysine and vernolepin at pH 7.4 was allowed to stand for 6 days at 25°C, 75% of the original lactone could be recovered. Similarly, guanine proved unreactive toward either vernolepin or elephantopin. The biscysteine adducts, as expected, were essentially inactive (9).

Structure-cytotoxicity relationships of elephantopin derivatives (fig 6) paralleled those of vernolepin. Similarly, the reactivity of the functional groups toward cysteine (fig 7) paralleled those of vernolepin. More recent studies of several dozen cytotoxic sesquiterpene lactones confirmed the requirement

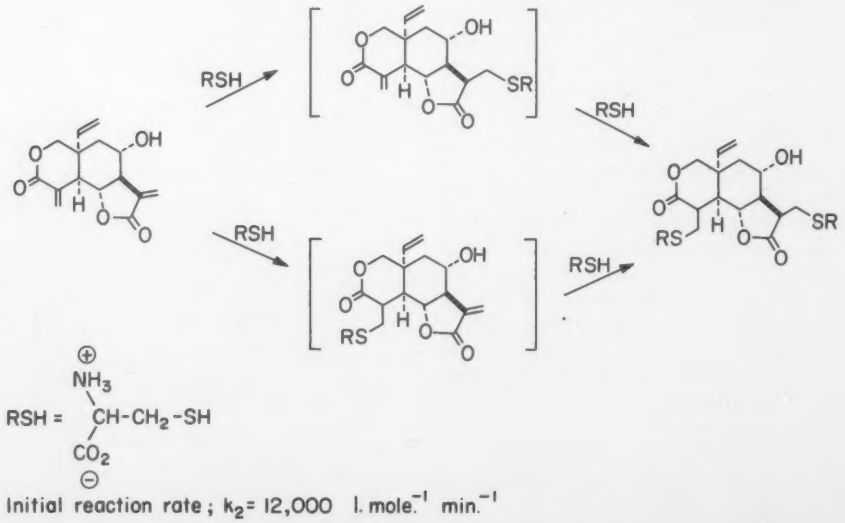


FIGURE 5.—Reaction of vernolepin with L-cysteine at 25°C, pH 7.4.

for an α-methylene-γ-lactone for cytotoxicity and pointed to the need for a second unsaturated carbonyl function for in vivo tumor-inhibitory activity (10).

Systematic studies of a tumor-inhibitory extract from *Taxodium distichum* Rich led to isolation and structural elucidation of two novel diterpenoid quinone methides, taxodione *1* and taxodone *5* (fig 8) (11,12). The structures were deduced from their formulae and spectra, and confirmed by interrelation with a known diterpene. Both quinone methides were converted to *2*, which was methylated to *3*. Reduction of *3* with lithium aluminum hydride gave the 6β-alcohol *4*, which was dehydrated to *6*. Catalytic hydrogenation of *6* gave *7*, characterized by direct comparison with a sample prepared from sugiol (*8*). Among the six diterpenoid derivatives isolated from *T. distichum* only the quinone-methide derivatives taxodione and taxodone showed significant inhibitory activity in vivo against the Walker 256 carcinosarcoma in the rat and in vitro against cells derived from human carcinoma of the nasopharynx (KB). This fact, and the known sensitivity of quinone methides to nucleophilic attack, suggested that taxodione and taxodone might exert their biologic effect by interaction with a biologic nucleophile at carbon 7.

Extracts of *Jatropha gossypiifolia* have been used to treat cancer in Costa Rica and elsewhere for

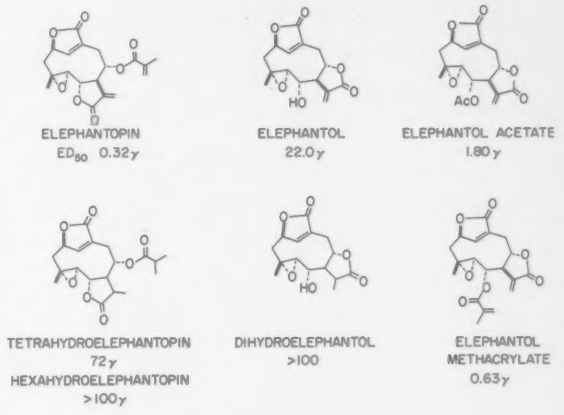


FIGURE 6.—Cytotoxicity of elephantopin derivatives.

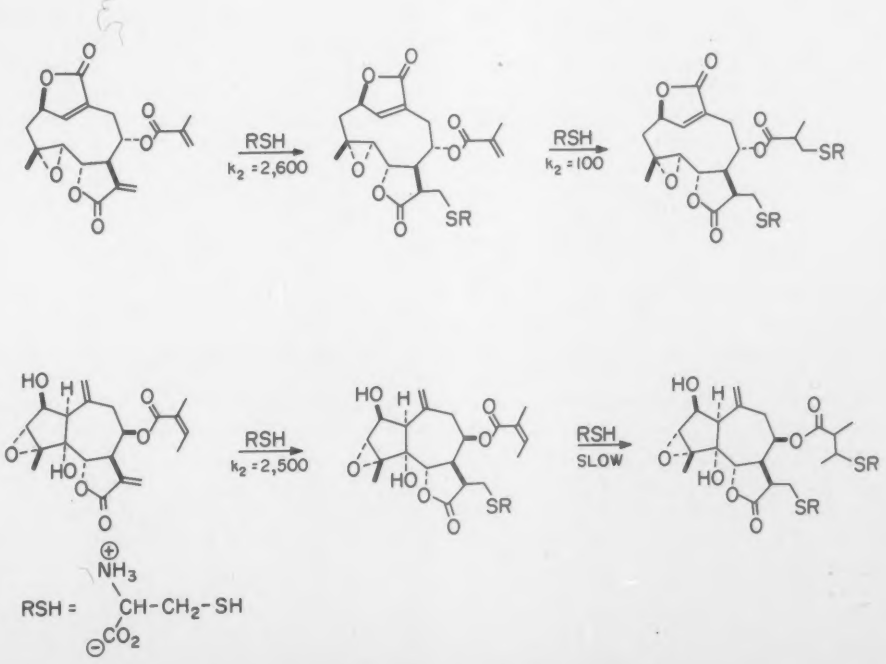


FIGURE 7.—L-Cysteine adduct formation at 25°C, pH 7.4.

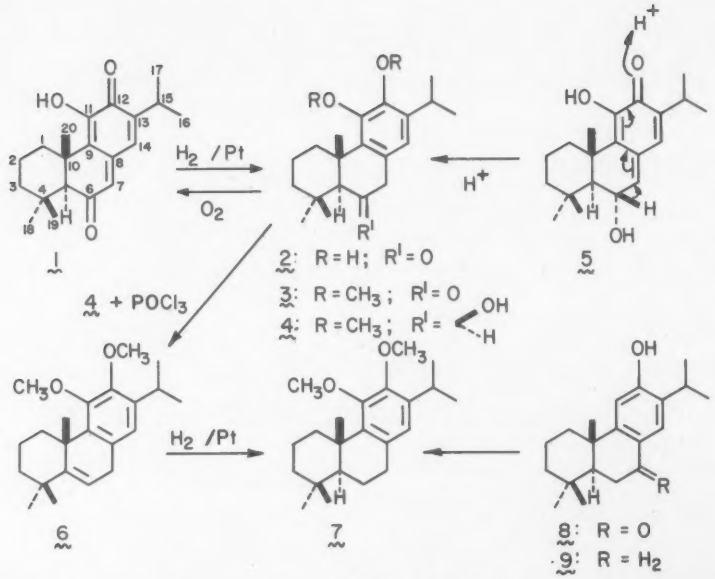


FIGURE 8.—Taxodione and taxodone: principles of *Taxodium distichum* which inhibit growth of Walker 256 carcinosarcoma.

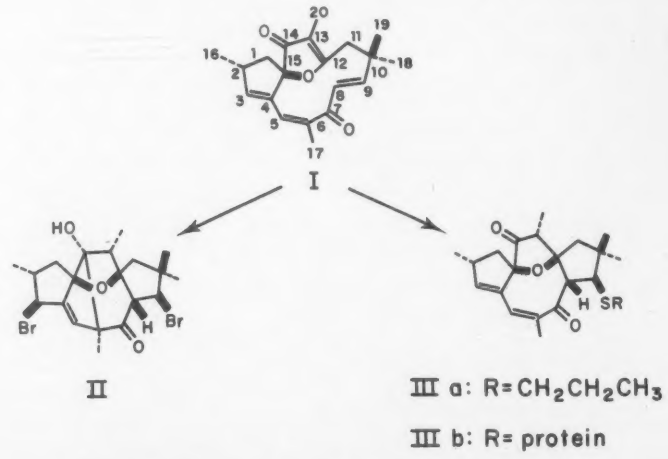
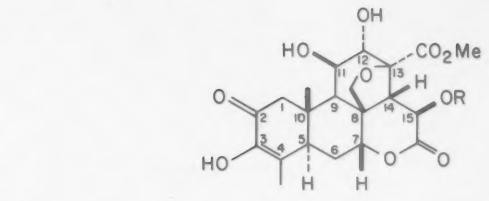
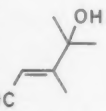


FIGURE 9.—Products of nucleophilic addition to jatrophone.

many years. When an extract forwarded by Professor J. A. Saenz Renauld was tested by the NCI, the extract was found to inhibit five standard tumor systems. Systematic fractionation led to isolation of the novel antileukemic macrocyclic diterpenoid jatrophone (I), whose structure was elucidated with the help of X-ray crystallographic analysis of the dihydrobromide adduct II (fig 9) (13). From the spectral properties of jatrophone and its reversible interrelation with II, structure I, with uncertainty only about the stereochemistry of the 5,6-double bond, could be derived for jatrophone. The *cis*-configuration of the 5,6-double bond and the absolute configuration of jatrophone were established by direct single-crystal X-ray analysis (14). To evaluate whether jatrophone's electrophilicity would be manifested in reactions with model biologic nucleophiles, the compound was treated with *n*-propyl thiol. A well-characterized thiol monoadduct (fig 9, IIIa) was obtained (14).



	KB (ED_{50}; $\mu g/m\ell$)
Bruceantin : R = OC	(4.9×10^{-3})
Bruceantarin : R = OCC_6H_5	(3.7×10^{-2})
Bruceine B : R = $OCCH_3$	(2.8×10^{-1})
Bruceolide : R = H	(> 10)



Bruceantinol : R = OC	(2.2×10^{-3})
Dihydrobruceantin : R = $OCCH_2\ CH(CH_3)CH(CH_3)_2$	(5.5×10^{-3})

FIGURE 10.—Active principles of *Brucea antidysenterica*.

Brucea antidysenterica Mill. is a simaroubaceous tree which is used in Ethiopia in the treatment of cancer. We found that an alcoholic extract of the plant showed significant inhibitory activity in vitro against cells derived from cancer of the nasopharynx and against two standard animal tumor systems. Systematic fractionation led to isolation and structural elucidation of three new bitter principles, bruceantin, bruceantinol, and bruceantarin (fig 10) (15,16). Bruceantin and bruceantinol show potent antileukemic activity at microgram/kilogram dose levels and over a wide dosage range. In contrast, bruceantarin and bruceine B, which differ only in the ester moiety, show diminished antileukemic activity. It seems likely that the markedly higher potency of bruceantin and bruceantinol may be attributable to the role of the α,β-unsaturated esters. Bruceantin has been extracted on a large scale for toxicologic investigation in preparation for clinical trials by the NCI.

An ethanolic extract of *Acer negundo* L. was shown to possess significant inhibitory activity against the Sarcoma 180 and the im Walker 256 carcinosarcoma tumor systems. Systematic fractionation led to the isolation of the active principles as the chromatographically homogeneous acids, saponin P and saponin Q (17). Saponin P has shown a high therapeutic index in further biologic studies, and the NCI has procured a large collection of *Acer negundo* for extraction of saponin P in a quantity sufficient for preclinical toxicologic studies and preliminary clinical trials. Our chemical studies of saponin P began with acid hydrolysis, which yielded glucose and arabinose, along with two aglycones, acerotin and acerocin (fig 11). We have recently shown that the aglycones are diesters of the new acidic triterpene acerogenic acid. Each has a unique nonadienoate residue; one has *trans-trans* stereochemistry and the other has *cis-trans* stereochemistry (18). As the presence of α,β-unsaturated carbonyl functions has been shown to be responsible for the tumor-inhibitory activity of other natural products, the electrophilic unsaturated esters in saponin P appear to contribute significantly in making this material the most promising of the known tumor-inhibitory saponins.

An alcoholic extract of *Tripterygium wilfordii* Hook was found to show significant activity against the L1210 leukemia in mice. Systematic fractionation led to isolation of triptolide (*1*) and tripdiolide (*2*) (fig 12) as the antileukemic principles, and direct X-ray crystallographic analysis resulted in elucidation of the structures (19). The diepoxide functionality has been shown to confer tumor-inhibitory activity on certain classes of acyclic synthetic compounds (2), as well as the naturally occurring cyclohexane diepoxide, crotepoxide (20). The α,β-unsaturated lactone function has been shown to be important for

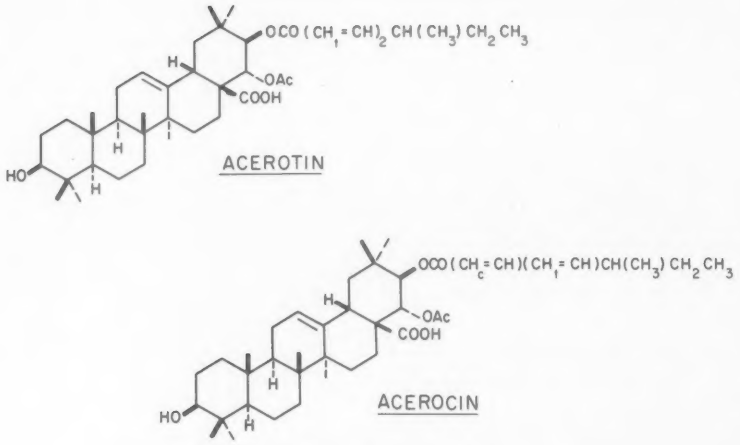


FIGURE 11.—Structures of acerotin and acerocin.

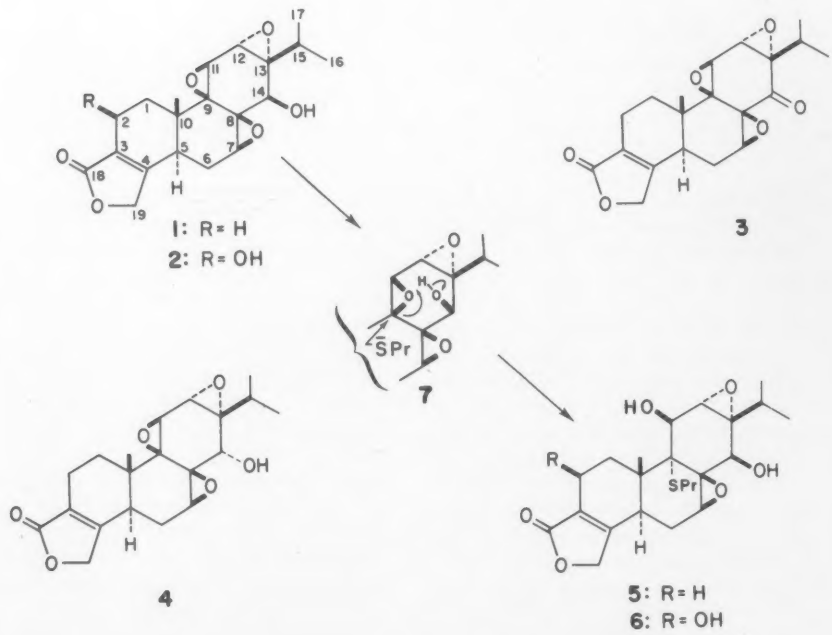


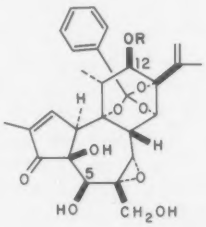
FIGURE 12.—Structures of the triptolides and their derivatives.

the tumor-inhibitory activity of several classes of terpenoids (21). Consequently, we hypothesized that one or more of the four highly electrophilic functions of the antileukemic triptolides *1* and *2* may be involved in their mode of action. The NMR spectra of *1* and *2* display resonances attributable to strong hydrogen bonding between the 14-hydroxyl and the 9,11-epoxide groups (22). The co-occurring keto-analog triptonide (3), which differs from *1* solely at carbon 14, shows neither the resonances attributable to the hydrogen-bonded epoxide system nor the antileukemic activity. Subsequent testing of the minor variants *4*, *5*, and *6* revealed that these triptolide derivatives also do not show antileukemic activity. On treatment with propanethiol, *1* and *2* underwent selective nucleophilic attack at carbon 9 to yield the

adducts *5* and *6*, respectively. In contrast, *4* was recovered largely unchanged after similar treatment. The increased reactivity of the antileukemic triptolides *1* and *2*, relative to the inactive 14-epimeric derivative *4*, may be attributable to hydroxyl-group participation in the epoxide opening of the former compounds, as depicted in *7*. The results agree with the hypothesis that the hydroxyl-assisted attack by nucleophiles on the 9,11-epoxide may mimic the mechanism by which the antileukemic triptolides exert their biologic activity (22). Conceivably, in vivo, one or more of the additional electrophilic functions may effect subsequent alkylation of other neighboring nucleophiles on the biologic macromolecule.

An ethanol extract of *Gnidia lamprantha* Gilg. showed a high order of inhibitory activity against the P388 leukemia in mice. Systematic fractionation, guided initially by P388 assay and later by a combination of this and goldfish toxicity tests, led to the isolation of three potent antileukemic principles. Structural studies revealed that gnididin, gniditrin, and gnidicin possess the closely related structures depicted in figure 13 (23). A similar study of *Daphne mezereum* L. (also of the family Thymelaeaceae, and widely used in folk medicine for treating cancers) led to isolation and characterization of mezerein (fig 13) as the principal antileukemic component (24).

Alcoholic extracts of *Maytenus serrata* (formerly known as *M. ovatus*) showed significant activity against five standard animal tumor systems in the NCI's screen. Fractionation of the alcoholic extract was guided by assay for activity against KB cells in vitro or P388 leukemia in mice. A highly



GNIDIDIN : R = COCH = CHCH = CH$(CH_2)_4CH_3$

GNIDITRIN : R = COCH = CH$(CH=CH)_2(CH_2)_2CH_3$

GNIDICIN : R = COCH = CHC_6H_5

MEZEREIN : R = COCH = CHCH = CHC_6H_5

FIGURE 13.—Antileukemic principles of *Gnidia* and *Daphne* species.

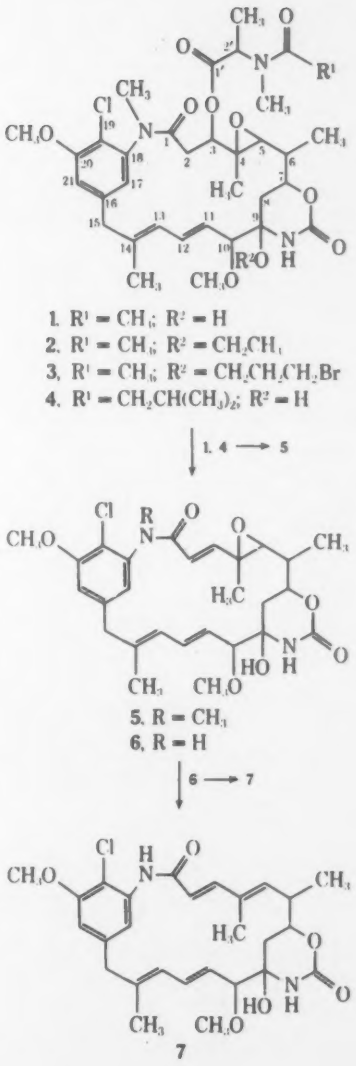


FIGURE 14.—Structures and interrelations of maytansinoids.

enriched concentrate was prepared by solvent partitions, column chromatography, acetylation, and further column chromatography and repeated preparative thin-layer chromatography. Maytansine (3-bromopropyl) ether was prepared from the concentrate and was characterized by X-ray crystallographic analysis as *3* in figure 14 (cf, fig 15) (25). Hydrolysis of the derivative yielded crystalline maytansine (*1*), which was used to seed the concentrate to yield additional maytansine. Maytansine is an exceptionally interesting antitumor agent, for it is active at the level of micrograms/kilogram of animal

body weight. Furthermore, it exerts significant inhibitory activity against the mouse P388 lymphocytic leukemia over a 50–100-fold dosage range. Interest was heightened by the finding that maytansine also shows significant inhibitory activity against the L1210 mouse leukemia and the Lewis lung carcinoma and B16 melanoma solid murine tumor systems. After extensive preclinical toxicologic studies, maytansine was recently selected for clinical trial by the NCI.

Recent studies have led to the isolation and characterization of four new maytansinoids from *M. buchananii* (26). Maytanvaline (*4*) is a highly active antileukemic maytanside ester. Maysine (*5*), normaysine (*6*), and maysenine (*7*) lack antileukemic activity, and show about 1/10,000th the cytotoxicity of maytanside esters such as maytansine (*1*). The X-ray crystallographic structural elucidation of maytansine (3-bromopropyl) ether (*3*) revealed that the two longer sides of the 19-membered ring are roughly parallel and separated by about 5.4 angstroms, so that there is a hole in the center of the ring (cf, fig 15) (25,27). The two faces of the ring have a different character; the lower surface, opposite the ester residue, is predominantly hydrophobic, whereas the upper face is more hydrophilic. Furthermore, the ester residue is oriented in a manner which would hinder sterically the approach of reactants to the hydrophilic face. The ester function in the antileukemic maytansinoids may play a key role in the formation of highly selective molecular complexes with growth-regulatory biologic macromolecules. Such molecular complex formation may be crucial for the subsequent selective alkylation of specific nucleophiles, by, for example, the carbinolamide and epoxide functions. In this connection, it is noteworthy that maytansine ethyl ether (*2*), in which the reactive carbinolamide is no longer available as a potential alkylating function, shows no antileukemic activity.

Recent studies have led to the isolation, structural elucidation, and chemical interrelation of two new maytansinoids from *Putterlickia verrucosa* Szyszyl., the richest reported source of maytansine and related antileukemic esters (28). Maytanacine, which bears an acetate group at carbon 3, is a potent antileukemic compound, and is the first reported maytanside ester which does not bear an amino acid residue at carbon 3. Maytansinol, which bears a hydroxyl group at carbon 3, lacks antileukemic activity and shows about 1/100,000th the cytotoxicity of maytanacine.

BIOCHEMISTRY OF THE NEW TUMOR INHIBITORS

To test the possibility that the quinone methides, taxodone and taxodione, and α-methylene lactones, such as vernolepin, may react with sulfhydryl enzymes, we studied their effects on phosphofructokinase (29). Phosphofructokinase contains 16–18 sulfhydryl groups per protomer of 93,200 daltons. The tumor inhibitors were found to inhibit phos-

FIGURE 15.—Molecular structure of maytansine (3-bromopropyl) ether.

phofructokinase, and evidence was presented that the inhibition results from their reaction with the sulfhydryl groups of the enzyme.

Taxodione was the most potent of the tumor inhibitors in diminishing phosphofructokinase activity. An addition of only 1.6 mols of taxodione per protomer of enzyme reduced activity by 50%. The other quinone methide, taxodone, inhibited 50% at a relative concentration of 32, and vernolepin and other sesquiterpene lactones inhibited 50% at relative concentrations of 1000–2000.

Similar results were obtained in a study of the action of vernolepin on the enzyme glycogen synthase (30). Vernolepin inactivates glycogen synthase. To measure the stoichiometry of the reaction with the enzyme protein, a procedure for tritiation of vernolepin was developed. Reaction with 3 mols of radioactive reagent per 90,000-dalton subunit caused virtually complete loss of activity. The concurrent disappearance of three titratable thiol groups (out of six) indicated that thioether formation (ie, alkylation of sulfhydryl groups) is the major mode of binding to the protein. The persistence of one or two residual thiol groups in the enzyme subunit, even after incubation with vernolepin in the presence of sodium dodecyl sulfate, pointed up the selectivity in the reaction with vernolepin.

In a recent study, jatrophone was shown to react with small molecular weight thiols as well as thiol groups on proteins such as bovine serum albumin and DNA-dependent RNA polymerase from *Escherichia coli* (31). When bovine serum albumin was treated with jatrophone, all of the sulfhydryl groups in the albumin molecule had reacted after 15 hours. Gel filtration afforded a good separation between protein and excess jatrophone. The separated protein contained virtually no free sulfhydryl groups, and the ultraviolet spectrum was in agreement with a monoadduct product of type IIIb (fig 9). When a portion of the jatrophone-treated protein was precipitated with trichloroacetic acid, no absorbing material was detected in the supernatant, indicative that the jatrophone was covalently linked to the albumin.

The reaction between jatrophone and protein sulfhydryl groups was further investigated with RNA polymerase from *E. coli* (31). After 4 hours, approximately 50% of the activity had been lost, and approximately eight sulfhydryl groups on the enzyme had reacted with jatrophone. Further incubation up to 22 hours resulted in almost complete loss of activity. At that point, 11 sulfhydryl groups of the enzyme had reacted. When a sample of the polymerase which had been allowed to react with jatrophone for 4 hours was subjected to gel filtration, the spectrum of the treated polymerase was similar to that of jatrophone-treated bovine serum albumin. Evidently, in this case also, a monoadduct of type IIIb had been formed.

A study of the mode of action of bruceantin revealed that the compound irreversibly inhibits protein synthesis in HeLa cells, rabbit reticulocytes, and reticulocyte lysates (32). Furthermore, the results suggested that the principal inhibitory effect of the drug is on initiation of protein synthesis. The structure-activity relationships of bruceantin and its analogs correlated well with their in vivo activity as antitumor agents in experimental animals. The studies demonstrated that the ester side chain of bruceantin is important for transport of the drug into intact cells, and that the intact diosphenol system in ring A is required for inhibition of protein synthesis.

Maytansine, at 6×10^{-8} M, has been shown to inhibit, irreversibly, cell division in eggs of sea urchins and clams (33). It causes the disappearance of a mitotic apparatus or prevents one from forming if added at early stages. Maytansine does not affect formation of the mitotic organizing center but does inhibit in vitro polymerization of tubulin. Maytansine and vincristine inhibit in vitro polymerization of tubulin at about the same concentrations, but vincristine is about 100 times less effective as an inhibitor of cleavage in marine eggs. It was found that maytanbutine (34), a homologous maytanside ester, readily alkylated *n*-propane thiol to form the 9-*n*-propyl thioether. Presumably, maytansine may alkylate selected protein thiols in an analogous manner.

CONCLUSION

The activity-directed isolation of new tumor inhibitors of plant origin has yielded many novel compounds with significant growth-inhibitory properties. A large proportion of the new compounds contain highly electrophilic functionalities, and chemical and biochemical studies support the view that these compounds may act by selective alkylation of growth-regulatory biologic macromolecules. The selectivity may result from many factors, among which are transport of the tumor inhibitor into the cell and the chemical nature and steric environment of the specific nucleophile to be alkylated. Model studies support the hypothesis that the inhibition of tumor growth by the new agents may be attributable to selective alkylation of key enzymes which control cell division. The new tumor inhibitors may have a triple selectivity (*a*) generally for thiols over other nucleophiles, (*b*) specifically for particular sulfhydryl enzymes, and (*c*) specifically for particular sulfhydryl groups within those enzymes. The defini-

tive characterization of the nature of the nucleophiles and of the biologic macromolecules which bear these nucleophiles is among the goals at the very frontier of research in cancer chemotherapy.

REFERENCES

1. GILMAN A, and PHILIPS FS. The biological actions and therapeutic applications of the β-chloroethyl amines and sulfides. Science 103:409–415, 1946.
2. ROSS WCJ. Biological alkylating agents. London, Butterworth, 1962.
3. WHEELER GP. Studies related to the mechanisms of action of cytotoxic alkylating agents: a review. Cancer Res 22:651–688, 1962.
4. KUPCHAN SM, AYNEHCHI Y, CASSADY JM, ET AL. The isolation and structural elucidation of two novel sesquiterpenoid tumor inhibitors from *Elephantopus elatus*. J Am Chem Soc 88:3674–3676, 1966.
5. KUPCHAN SM, AYNEHCHI Y, CASSADY JM, ET AL. Tumor inhibitors. XL. The isolation and structural elucidation of elephantin and elephantopin, two novel sesquiterpenoid tumor inhibitors from *Elephantopus elatus*. J Org Chem 34:3867–3875, 1969.
6. KUPCHAN SM, HEMINGWAY RJ, WERNER D, ET AL. Tumor inhibitors. Vernolepin, a novel elemanolide dilactone tumor inhibitor from *Vernonia hymenolepis*. J Am Chem Soc 90:3596–3597, 1968.
7. KUPCHAN SM, HEMINGWAY RJ, WERNER D, ET AL. Tumor inhibitors. XLVI. Vernolepin, a novel sesquiterpene dilactone tumor inhibitor from *Vernonia hymenolepis* A. Rich. J Org Chem 34:3903–3907, 1969.
8. SEQUEIRA L, HEMINGWAY RJ, and KUPCHAN SM. Vernolepin: a new, reversible plant growth inhibitor. Science 161:789–790, 1968.
9. KUPCHAN SM, FESSLER DC, EAKIN MA, ET AL. Reactions of alpha methylene lactone tumor inhibitors with model biological nucleophiles. Science 168:376–378, 1970.
10. KUPCHAN SM, EAKIN MA, and THOMAS AM. Tumor inhibitors. 69. Structure-cytotoxicity relationships among the sesquiterpene lactones. J Med Chem 14:1147–1152, 1971.
11. KUPCHAN SM, KARIM A, and MARCKS C. Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from *Taxodium distichum*. J Am Chem Soc 90:5923–5924, 1968.
12. KUPCHAN SM, KARIM A, and MARCKS C. Tumor inhibitors. XLVIII. Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from *Taxodium distichum*. J Org Chem 34:3912–3918, 1969.
13. KUPCHAN SM, SIGEL CW, MATZ MJ, ET AL. Jatrophone, a novel macrocyclic diterpenoid tumor inhibitor from *Jatropha gossypiifolia*. J Am Chem Soc 92:4476–4477, 1970.
14. KUPCHAN SM, SIGEL CW, MATZ MJ, ET AL. Structure and stereochemistry of jatrophone, a novel macrocyclic diterpenoid tumor inhibitor. J Am Chem Soc. In press.
15. KUPCHAN SM, BRITTON RW, ZIEGLER MF, ET AL. Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J Org Chem 38:178–179, 1973.
16. KUPCHAN SM, BRITTON RW, LACADIE JA, ET AL. The isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from *Brucea antidysenterica*. J Org Chem 40:648–654, 1975.
17. KUPCHAN SM, HEMINGWAY RJ, KNOX JR, ET AL. Tumor inhibitors. XXI. Active principles of *Acer negundo* and *Cyclamen persicum*. J Pharm Sci 56:603–608, 1967.
18. KUPCHAN SM, TAKASUGI M, SMITH RM, ET AL. Tumor inhibitors. LXII. The structures of acerotin and acerocin, novel triterpene ester aglycones from the tumor inhibitory saponins of *Acer negundo*. J Org Chem 36:1972–1976, 1971.
19. KUPCHAN SM, COURT WA, DAILEY RG, JR, ET AL. Triptolide and tripdiolide, novel antileukemic diterpenoid triepoxides from *Tripterygium wilfordii*. J Am Chem Soc 94:7194–7195, 1972.
20. KUPCHAN SM, HEMINGWAY RJ, COGGON P, ET AL. Crotepoxide, a novel cyclohexane diepoxide tumor inhibitor from *Croton macrostachys*. J Am Chem Soc 90:2982–2983, 1968.
21. KUPCHAN SM. Recent advances in the chemistry of terpenoid tumor inhibitors. Pure Appl Chem 21:227–246, 1970.
22. KUPCHAN SM, and SCHUBERT RM. Selective alkylation: a biomimetic reaction of the antileukemic triptolides? Science 185:791–793, 1974.
23. KUPCHAN SM, SWEENY JG, BAXTER RL, ET AL. Letter: Gnididin, gniditrin, and gnidicin, novel potent antileukemic diterpenoid esters from *Gnidia lamprantha*. J Am Chem Soc 97:672–673, 1975.
24. KUPCHAN SM, and BAXTER RL. Mezerein: antileukemic principle isolated from *Daphne mezereum* L. Science 187:652–653, 1975.
25. KUPCHAN SM, KOMODA Y, COURT WA, ET AL. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J Am Chem Soc 94:1354–1356, 1972.
26. KUPCHAN SM, KOMODA Y, BRANFMAN AR, ET AL. Novel maytansinoids. Structural interrelations and requirements for antileukemic activity. J Am Chem Soc 96:3706–3708, 1974.
27. BRYAN RF, GILMORE CJ, and HALTIWANGER RC. Crystal and molecular structure and absolute configuration of maytansine (3-bromopropyl) ether. J Chem Soc [Perkin Trans II], 1973, pp 897–902.
28. KUPCHAN SM, BRANFMAN AR, SNEDEN AT, ET AL. Letter: Novel maytansinoids. Naturally occurring and synthetic antileukemic esters of maytansinol. J Am Chem Soc 97:5294–5295, 1975.
29. HANSON RL, LARDY HA, and KUPCHAN SM. Inhibition of phosphofructokinase by quinone methide and α-methylene lactone tumor inhibitors. Science 168:378–380, 1970.
30. SMITH CH, LARNER J, THOMAS AM, ET AL. Inactivation of glycogen synthase by the tumor inhibitor vernolepin. Biochim Biophys Acta 276:94–104, 1972.
31. LILLEHAUG JR, KLEPPE K, SIGEL CW, ET AL. Reaction of biological thiols with the tumor inhibitor jatrophone. Inhibition of RNA polymerase. Biochim Biophys Acta 327:92–100, 1973.
32. LIAO L-L, KUPCHAN SM, and HORWITZ SB. Mode of action of the antitumor compound bruceantin, an inhibitor of protein synthesis. Mol Pharmacol 12:167–176, 1976.
33. REMILLARD SP, REBHUN LI, HOWIE GA, ET AL. Antimitotic activity of the potent tumor inhibitor maytansine. Science 189:1002–1005, 1975.
34. KUPCHAN SM, KOMODA Y, THOMAS GJ, ET AL. Maytanprine and maytanbutine, new antileukaemic ansa macrolides from *Maytenus buchananii*. J C S Chem Commun, 1972, p 1065.

Pharmacology of Antitumor Agents From Higher Plants

Susan M. Sieber, J. A. R. Mead, and Richard H. Adamson[1,*]

SUMMARY

Some pharmacologic properties of nine antitumor agents from higher plants are described. The agents are vincristine, vinblastine, the epipodophyllotoxin derivatives VM-26 and VP-16-213, maytansine, bruceantin, thalicarpine, camptothecin, and lapachol. When sufficient information is available, the agents are discussed with regard to their antitumor activity, mechanism of action, pharmacologic disposition, structure-activity relationships, and toxicity.

[Cancer Treat Rep 60:1127–1139, 1976]

During the past 20 years, an increasingly intensive search has been under way for chemicals with antitumor activity. Plants are a particularly interesting source of such substances, since they provide chemicals with novel structures not likely to be synthesized in the laboratory. Thus far, many plant products have been found to be active in animal tumor systems, and a few are also effective inhibitors of the growth of some human tumors. Structural elucidation of such substances has, in some instances, permitted the systematic testing of structural analogs in attempts to obtain drugs with greater antitumor activity and less toxicity. Some of the plant-derived antitumor agents considered here, such as vincristine and vinblastine, are well established in clinical practice. Two epipodophyllotoxin derivatives, VM-26 and VP-16-213 (VP-16), have definite clinical activity but their value in cancer treatment has not yet been well defined. Preclinical toxicologic evaluation of two relatively new agents is completed (maytansine) or nearly completed (bruceantin); three other agents (lapachol, thalicarpine, and camptothecin) possess antitumor activity in animal tumor systems, but enthusiasm for their use waned after phase I clinical trials showed major toxic complications with little or no therapeutic benefit. Before describing the pharmacology of these agents, some aspects of pharmacologic studies relating to antitumor drugs in general should be considered.

In general, plant products evaluated for antitumor effects are extracted into alcohol and the crude extracts are subjected to a systematic fractionation procedure which is guided by bioassay. The fractions are tested for in vitro activity against human KB cells or for in vivo activity against murine P388 leukemia. If repeated testing confirms the presence of antitumor activity in one or more fractions, attempts are initiated to isolate the active component. After the pure compound is isolated, it is tested in other animal tumor systems including L1210 leukemia, B16 melanoma, and Lewis lung carcinoma (1). When activity against these tumors is confirmed in a second laboratory, the new compound can be considered for pharmacologic evaluation.

The criteria used in selecting new compounds for pharmacologic evaluation include chemical structure, degree and spectrum of activity against the four tumor systems mentioned above, and therapeutic index (the ratio between the minimum effective dose and the maximum tolerated dose). The compound must have a unique chemical structure, differing significantly from compounds previously evaluated. Ideally, it is active against both L1210 and P388 leukemias and the solid tumors, B16 melanoma and Lewis lung carcinoma, inducing ≥ 50% increases in the survival of animals with L1210 leukemia, B16 melanoma, and Lewis lung carcinoma; the lifespan of animals with P388 leukemia should be prolonged by 75% or more. As a general rule, the new compound must be active against at least one leukemia and one solid tumor. Selection of a drug for pharmacologic evaluation is favored if it has a moderate to high therapeutic index.

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Pharmacologic evaluation of selected compounds follows two avenues: (*a*) preclinical toxicology, and (*b*) general pharmacology. A major goal of preclinical toxicology is to establish a safe starting dose for use in clinical trials. These studies also provide information as to the major organ toxicity peculiar to the drug under investigation; such toxicity should be both predictable and reversible. These studies are performed in normal dogs and monkeys and include determination of (*a*) the "toxic dose low," the dose at which there is minimal change in the function of vital organs and which when doubled is not lethal, (*b*) the "toxic dose high," the dose at which there is marked change in the function of vital organs and which when doubled is lethal, and (*c*) the lethal dose. In addition, the optimum therapeutic dose and the toxic dose must be established in tumor-bearing mice. The information developed during preclinical toxicology is an essential element of the investigational new drug (IND) application filed with the Food and Drug Administration to gain approval for clinical evaluation of the drug.

General pharmacologic evaluation of a new drug involves a broad array of studies designed to provide information about its pharmacokinetics, mechanism of action, and structure-activity relationships, all of which are useful in guiding further preclinical and clinical evaluation. These studies are directed toward determining dose-response relationships, optimal route of administration, most effective dose and dose schedule, and effectiveness of drug combinations. Parallel studies are carried out on the physiologic disposition of the drug, eg, its absorption, distribution, metabolism, and excretion, and on its degree of binding to plasma proteins. These studies are, of course, contingent upon developing a sufficiently sensitive and specific method for measuring the drug and its metabolites in biologic material. The availability of radioactively labeled drugs has greatly facilitated studies of their physiologic disposition in animals. Information on metabolism and subsequent excretory pattern is of particular importance, since serious and unexpected toxicity can arise from accumulation of a drug normally detoxified by the liver and/or excreted by the kidney in patients with compromised hepatic or renal function.

Orally administered drugs which are well absorbed from the gastrointestinal tract, as well as drugs given iv, reach their appropriate target site via the blood. They are carried in the blood in either a free state or bound to plasma protein. Since protein-bound drug is pharmacologically inactive, it is important to determine the extent to which drugs bind to plasma protein. The rate at which a drug reaches its target site as well as other nontarget organs and tissues is primarily a function of blood flow. The concept of C × T, the product of concentration and time, has been applied to antitumor agents in an attempt to quantitate the length of time that an effective drug concentration must be maintained at the target site for optimal antitumor effect (2). The plasma half-life of a drug is the time required for its concentration in plasma to decrease by 50%. Plasma half-life gives a suggestion of drug availability to target cells and therefore can be useful in determining optimal dosage schedules. However, this is not a simple correlation and it should not be rigidly applied in the absence of information on the other pharmacokinetic factors affecting drug action. A knowledge of the drug dosage required to produce an effective plasma level, as well as an estimate of the plasma half-life of the drug, are valuable in devising effective treatment schedules and in minimizing toxicity.

Information developed in these pharmacologic investigations provides an understanding of drug action and is used to guide further preclinical and clinical evaluation.

Alkaloids of *Vinca rosea* L.: Vincristine and Vinblastine

Although extracts of periwinkle, *Vinca rosea (Catharanthus roseus* [L] G. Don), were used as early as 1653 for hemostasis and for treating gingivitis and diabetes (3), it was > 300 years later that the leukopenic and antitumor activities of such extracts were noted. Indeed, it was the reported hypoglycemic activity of periwinkle extracts which led to the first detailed studies of their biologic effects. These studies demonstrated that a component of the extracts had myelosuppressive activity and could induce peripheral leukopenia; this component was soon isolated and termed vincaleucoblastine (vinblastine) (4). In another laboratory, workers found that periwinkle extracts had antileukemic activity; this led to the isolation of vincristine (5). Complete chemical characterization of vincristine was accomplished by Neuss et al (6).

Vincristine and vinblastine are structurally similar, differing from each other only in the substituent on the nitrogen atom in the vindoline molecule; thus vincristine possesses a formyl moiety in this position, and vinblastine possesses a methyl group (fig 1). Both compounds are complex structures composed of an indole nucleus (the catharanthine portion) linked to a dihydroindole nucleus (the vindoline portion). Structure-activity studies have defined certain features of these compounds which are essential for antitumor activity. For example, substitutions on the basic indole nitrogen of the catharan-

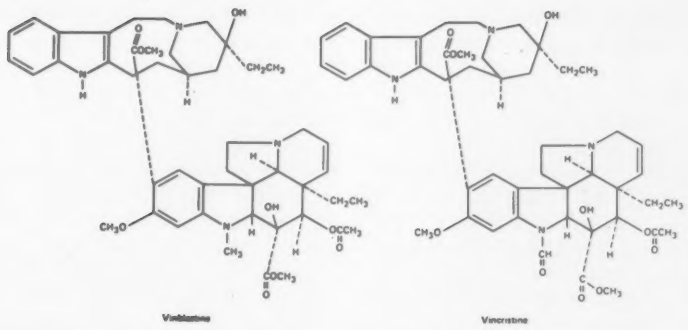


FIGURE 1

thine molecule lead to complete loss of antitumor activity (7); in addition, a substituent on the C-4 of the vindoline molecule is necessary for antitumor activity, although the substituent does not necessarily have to be an acetyl group (8). For vinblastine at least, acetylation of the free hydroxyl functions results in loss of antitumor activity (7).

In addition to their close structural similarity, vincristine and vinblastine are similar in regard to their spectrum of activity against experimental tumors. Although neither drug is effective in the murine L1210 leukemia system, both vincristine and vinblastine are active in several other animal tumor systems, including the murine P1534 and P388 leukemias and the Ridgway osteogenic sarcoma (9). Given by daily ip injection for 10 days after inoculation of tumor, vincristine is effective at doses of 0.2–0.4 mg/kg and vinblastine at doses of 0.4–0.6 mg/kg (10). Neither drug is effective when given orally, and there is no evidence that either vincristine or vinblastine crosses the blood-brain barrier. Cross resistance between vincristine and vinblastine has been reported for at least one animal tumor system, P815 leukemia.[2]

Although similar in their activity against experimental animal tumors, vincristine and vinblastine differ in their clinical spectrum of activity and in their toxic effects in man. Vinblastine is most widely used in disseminated Hodgkin's disease, and less frequently in neuroblastoma, Letterer-Siwe disease, choriocarcinoma, carcinoma of the testes, and in patients with metastatic breast cancer. Its most serious side effect in man is myelosuppression; other manifestations of its toxicity include paresis (usually mild and reversible), constipation, and alopecia. Vincristine is highly effective in acute lymphocytic leukemia, inducing a 90% response rate when administered in combination with corticosteroids. It is also used for treatment of Hodgkin's disease, Wilms' tumor, and rhabdomyosarcoma. Unlike vinblastine, vincristine does not depress bone marrow function; its major and dose-limiting adverse effect is peripheral neurotoxicity, manifested by paresthesias, loss of deep-tendon reflexes, paresis, and neurogenic constipation. Although these neurologic toxic manifestations are persistent, they are usually reversible (11).

Studies on the physiologic disposition of vincristine have been hampered by the lack of an analytic method sensitive enough to detect the low drug levels obtained after treatment with the therapeutic doses of vincristine. A radioactive form of the compound has only recently become available. Most studies directed at determining plasma levels of vincristine have utilized a bioassay method based on growth inhibition of tumor cells (KB, L, or P1534 cells) in culture. One such study demonstrated that after an iv dose of vincristine (1.0 mg/kg) to dogs and monkeys, the initial serum levels of 0.3–1.0 μg/ml decreased in 6 hours to 0.02–0.005 μg/ml. Rats given an ip injection of vincristine (2.0 mg/kg) showed maximum blood levels 20 minutes after dosing, although no drug was detectable in plasma 3 hours later. By contrast, maximum plasma levels in mice were attained by the earliest time interval tested (5 minutes after dosing), and low levels of drug were detectable 6 hours after treatment (12). In the only study of its physiologic disposition in man reported to date, children infused with vincristine (0.1 mg/kg in 1 hour) showed measurable blood levels of drug up to 4 hours after the end of the infusion (13).

Bleyer et al (14) examined the metabolism, uptake, and binding of vincristine by murine leukemia cells in vitro. They found that vincristine was not significantly metabolized by three different sublines of leukemia cells (L1210, P388, and a vincristine-resistant variant of P388) exposed to the drug for 1

[2]Burchenal J. Personal communication as cited in ref 9.

hour. In P388 cells, vincristine was rapidly accumulated by a carrier-mediated transport system and tightly bound, with the rate of transport slightly in excess of the rate of binding. Moreover, both transport and binding took place at a slower rate in the vincristine-resistant P388 leukemia cells (14).

There is somewhat more information with regard to the physiologic disposition of vinblastine in both animals and man, since a radioactive form of this drug has become available. Using tritiated vinblastine prepared by catalytic exchange, Creasey et al (15) examined the pharmacokinetics of vinblastine in dogs. They found a biphasic plasma-disappearance curve composed of an initial rapid phase (half-life of 17–38 minutes) followed by a more gradual decline (half-life of 3–5 hours). The amount of plasma tritium activity found in association with plasma proteins indicated that 28%–79% of the drug was protein bound. A relatively small proportion (12.1%–16.8%) of administered radioactivity was recovered in urine that was collected for 9 days after dosing. That the biliary pathway is the most important route of excretion was indicated not only by the recovery of 30%–36% of the tritium in the feces, but also by bile-plasma radioactivity ratios ranging from 7.3 to 56.9. One half or more of the bile radioactivity was identified as unchanged vinblastine (15). Qualitatively similar results were obtained in a study of the physiologic disposition of tritiated vinblastine in two cancer patients. Owellen and Hartke (16) found a biphasic plasma-disappearance curve, with half-lives of 4 minutes and 3 hours for the initial rapid and later slower phases, respectively. Approximately 20% of the administered radioactivity was recovered in urine collected for 3 days after dosing, of which 20%–50% was identified as unchanged vinblastine. A larger proportion of administered radioactivity (25%–41%) was recovered in the stool, although very little was attributable to unchanged vinblastine (16).

Despite numerous attempts to define the biochemical basis for their antitumor effects, little is known with regard to the mechanism of action of vincristine or vinblastine. Both compounds are mitotic inhibitors, causing metaphase arrest in dividing cells. It is thought (17) that this effect results from the interaction of vincristine and vinblastine with proteins of the spindle apparatus and the microtubules (tubulin). In fact, the formation of cytoplasmic inclusion bodies described as microtubule crystals has been noted in L-strain fibroblasts and human leukocytes exposed to vincristine or vinblastine (18) and it has been postulated that these crystals and the resultant disruption of the mitotic apparatus provide the basis for at least some of the antitumor activity of these compounds (15).

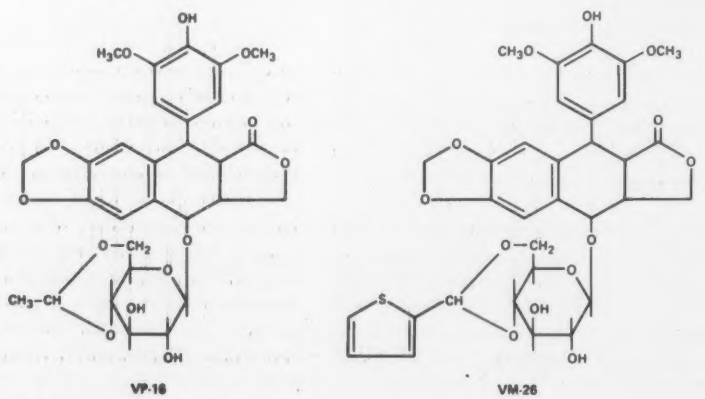


FIGURE 2

Lignan Derivatives of *Podophyllum peltatum* L.: VP-16 and VM-26

The biologic activity of the resin extracted from the roots and rhizomes of *Podophyllum peltatum* (mayapple or mandrake) was recognized as early as 1861, when Bentley (19) reported cytostatic activity in such extracts. Later studies showed that a component of this extract, podophyllin, was useful in treating condylomata acuminata (20). Chemical and pharmacologic examination of podophyllin revealed that its main active constituent was podophyllotoxin, and the isolation, purification, and chemical characterization of this substance led to a systematic search for its derivatives with less toxicity and more therapeutic efficacy (21). This search was carried out by various laboratories including Sandoz, Ltd (Basle, Switzerland) which produced numerous derivatives of podophyllotoxin. Two of the most promising of these semisynthetic podophyllotoxin derivatives tested to date are VP-16 and VM-26 (fig 2).

Both compounds possess antitumor activity against several experimental animal tumors including Sarcoma 180, Ehrlich ascites carcinoma, and Walker 256 carcinosarcoma; unlike most other plant-derived antitumor agents, they also have activity against L5178Y and L1210 murine leukemias (21–24). Both compounds are schedule dependent in most animal tumor systems, showing the greatest activity when given in two or three doses/week or every 3 hours on Days 1, 5, and 9 after tumor inoculation (22).

Information is accumulating as to the animal toxicity of these compounds. The LD17 in mice and rats given a single injection of VM-26 is approximately 50 mg/kg, but is reduced to about 5 mg/kg when the drug is given daily for 6–12 days (20). Avery et al (24) reported finding a severe form of delayed toxicity for VM-26 in mice carrying the L5178Y and L1210 tumors. Although the precise pathologic lesion responsible for this toxicity was not identified, there was some evidence that it was linked to the ip route of drug administration. Deaths from late toxicity occurred 1–3 months after the end of drug administration in 90%–100% of mice cured by treatment with the highest dose (16 mg/kg) of VM-26. In rhesus monkeys treated 6 days/week for 4 weeks with various doses of VM-26, the most serious toxic effects found were bone marrow suppression and colitis with diarrhea which were noted at doses of $\geq$ 0.4 mg/kg (25).

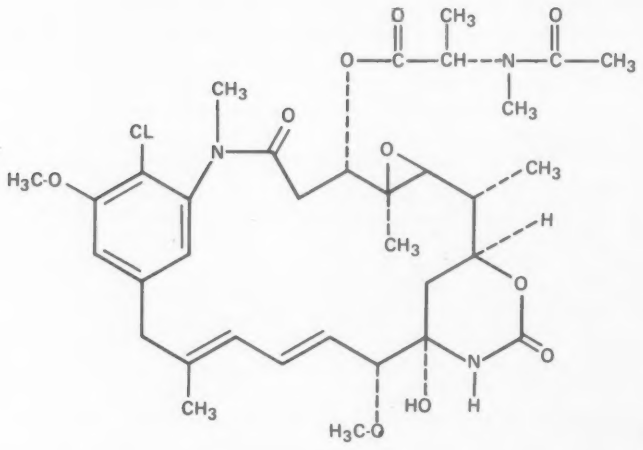
The clinical antitumor spectrum has not yet been well established for either VM-26 or VP-16. VM-26 has induced regressions and some complete remissions in Hodgkin's disease, in other lymphomas, and in bladder cancer (25–27). Although VP-16 is relatively less effective in these tumors, it has activity against acute myelogenous leukemia (28). VM-26 has also been reported to be effective in the treatment of intracranial malignancies (29).

In contrast to some similarities in their clinical spectra of activity, the pharmacokinetics of VM-26 and VP-16 differ in various respects. After administering the tritiated forms of these compounds to cancer patients, Allen and Creaven (30) found that VP-16 had a biphasic plasma-disappearance curve, whereas plasma-decay kinetics for VM-26 followed a triphasic exponential curve with a terminal phase half-life of 11–38 hours. Approximately 45% of the administered radioactivity was recovered in 72-hour urine of patients treated with either VM-26 or VP-16 of which 78% and 66%, respectively, were identified as parent compound. Although VP-16 resembled VM-26 in the extent to which it was excreted in the urine, its rate of excretion and renal clearance was some threefold greater than that of VM-26. Allen and Creaven (30) suggest that this difference may account for the threefold higher equitoxic dose of VP-16.

Both compounds show a high affinity for plasma proteins. At typical plasma concentrations, 94% of VP-16 was bound to serum albumin; for VM-26, this value was 99%. Neither compound penetrates the blood-brain barrier well since only low concentrations of the drugs were found in the cerebrospinal fluid; for VM-26 these levels were $< 1\%$ of those in plasma at corresponding times (31). Results of clinical trials performed to date suggest that the most troublesome form of toxicity of both compounds will be bone marrow suppression with peripheral leukopenia and thrombocytopenia (25,28,29).

Studies carried out to delineate the mechanism of action of VM-26 and VP-16 indicate that these agents have diverse actions at the biochemical level. In contrast to many other plant-derived compounds with antitumor activity (eg, podophyllotoxin itself, colchicine, and vinblastine) VM-26 and VP-16 do not arrest dividing cells in metaphase, but rather block cells in the premitotic stage of the cell cycle (22,23). This block, leading to a decrease in mitotic index, is due to inhibition of cells in the G_2 phase of the cell cycle (22). VP-16 incubated with various human hematopoietic cell lines inhibited cell growth and induced chromosomal aberrations in the G_2 and S phases of the cell cycle (32).

The preprophase effects of these compounds probably result in the inhibition of precursor incorporation into DNA, RNA, and protein noted by Misra and Roberts (33). Other effects of these compounds are the inhibition of respiration by Ehrlich ascites tumor cells and electron transport at the NADH



Maytansine

FIGURE 3

dehydrogenase level by mitochondria and submitochondrial particles from rat liver (34).

Ansa Macrolide of *Maytenus serrata* (Hochst. ex A. Rich.) R. Wilczek and *M. buchananii* (Loes.) R. Wilczek: Maytansine

The finding that an alcoholic extract from *Maytenus serrata* collected in Ethiopia possessed in vivo and in vitro antitumor activity led Kupchan's group to isolate and characterize its active principle, which they called maytansine (35). This compound was later isolated with higher yield from *M. buchananii* obtained in Kenya. Maytansine is an ansa macrolide (eg, an aromatic nucleus attached at two nonadjacent positions on the ring to a macrocyclic aliphatic bridge), with a structure reminiscent of the rifamycins and streptovaricins (fig 3). Although a number of maytansine derivatives have been isolated, structurally characterized, and tested for activity in experimental animal tumor systems (36), no clear structure-activity relationships have as yet emerged.

Maytansine has potent in vivo activity against experimental animal tumors including Sarcoma 180, Lewis lung carcinoma, B16 melanoma, and Walker 256 carcinosarcoma of the rat (35–38). Its antitumor activity is significant over a 50–100-fold dose range at the microgram/kilogram level. Maytansine also increases the survival of mice inoculated with vincristine-sensitive, but not vincristine-resistant, P388 leukemia cells; it suppresses the growth of L1210, L5178Y, and P388 leukemia cells in vitro and inhibits the incorporation of ^{3}H-thymidine into DNA in these cells. In addition, exposure to low (10^{-8}M) concentrations of maytansine causes a marked increase in the mitotic index of exponentially growing cultures of L1210 cells (37). An analysis of its stathmokinetic effects revealed that cells exposed to maytansine are arrested in metaphase, suggesting that the drug impairs the function of the mitotic spindle, in much the same manner as vincristine (37–39). A recent report has provided evidence that maytansine inhibits the in vitro polymerization of brain tubulin. Thus, it is possible that the antimitotic effects of this compound derive from its ability to interfere with tubulin polymerization in the mitotic apparatus (40).

Although little is known about the toxicity of maytansine, there is some evidence from animal studies that it depresses bone marrow and induces pancreatic and gastrointestinal lesions.[3] No data are as yet available concerning the physiologic disposition of this agent.

Quassinoid of *Brucea antidysenterica* J. F. Mill.: Bruceantin

Although principles from the simaroubaceous tree *Brucea antidysenterica* have been used in Ethiopia

[3]Preclinical toxicological evaluation of maytansine (NSC-153878) in dogs and monkeys. Battelle Columbus Laboratories, Ohio, May 1975, Report No. BCL-DTRDMN-153858-75-3 to the Laboratory of Toxicology, ETP, DCT, NCI.

for treating warts and condyloma acuminata (41), the first systematic investigation of such substances for antitumor activity was carried out by Kupchan et al (42). They showed that an ethanolic extract of this plant possessed antitumor activity against cultured human KB cells and prolonged the survival of mice inoculated with P388 leukemia and rats carrying the Walker 256 carcinosarcoma (42). The same group subsequently isolated and characterized the active constituent of the extract, bruceantin (fig 4).

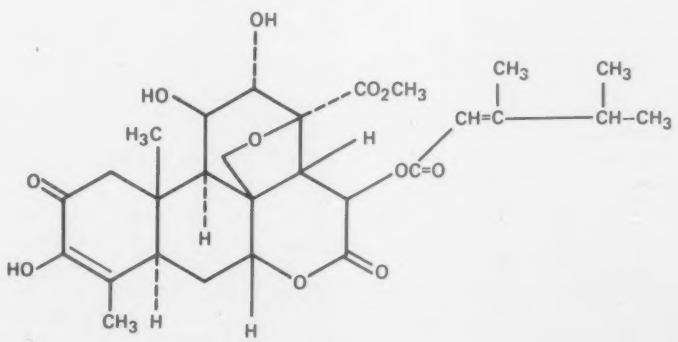
In addition to its antitumor effects against P388 leukemia and Walker 256 carcinosarcoma, bruceantin also has significant activity against L1210 leukemia, Lewis lung carcinoma, and B16 melanoma (43). Although relatively little information is available with regard to optimal antitumor dose levels and dosage schedules, preliminary results indicate that the most effective treatment regimen for all tumors involves daily treatment at a dose of 0.5–1.0 mg/kg. The mechanism of antitumor action of bruceantin is probably through an effect on protein synthesis. Thus Liao et al (44) demonstrated that, at a concentration of 5×10^{-8}M, bruceantin induces a 50% decrease in protein synthesis in cultured HeLa cells and in intact rabbit reticulocytes. A lesser effect on DNA synthesis, and no effect on RNA synthesis, was noted in these cells (44). Bruceantin-induced inhibition appears to be irreversible, and is most marked during initiation of protein synthesis (45). Studies on structure-activity relationships among bruceantin and various analogs indicate that the side chain of bruceantin is important for cellular uptake and that partial saturation of ring A is necessary for inhibition of protein synthesis (45).

At present there is no information available on either the toxicity or the physiologic disposition of bruceantin.

Alkaloid of *Thalictrum dasycarpum* Fisch., C. A. Mey. and Avé-Lall.: Thalicarpine

Thalicarpine was isolated from roots of *Thalictrum dasycarpum* by Kupchan et al (46), who noted its hypotensive effects in laboratory animals. In later studies with this compound, Kupchan reported that it had antitumor activity against the rat Walker 256 carcinosarcoma over a wide dosage range (47). Thalicarpine and several closely related compounds obtained from the same botanical source have also shown cytotoxic activity against human KB cells maintained in monolayer culture (48).

Thalicarpine is a dimeric alkaloid composed of an aporphine-benzylisoquinoline structure (fig 5). Although it possesses a high level of biologic activity in diverse systems, the mechanism by which it produces these effects is not clear. It has marked cardiovascular effects in dogs at a dose of 10 mg/kg, inducing hypotension and bradycardia, possibly through a nonspecific inhibition of smooth muscle activity (49). Similar cardiovascular effects were noted in monkeys treated with doses of > 2.5 mg/kg, and it was proposed that such effects were due to a combination of nonspecific vasodilation and direct myocardial depression (50). Allen and Creaven (51) have shown that thalicarpine has profound effects on macromolecular biosynthesis in cultured mouse L1210 leukemia cells, inhibiting DNA, RNA, and protein synthesis. Although DNA synthesis was affected more rapidly than either RNA or protein synthesis, ultraviolet spectral analysis did not indi-

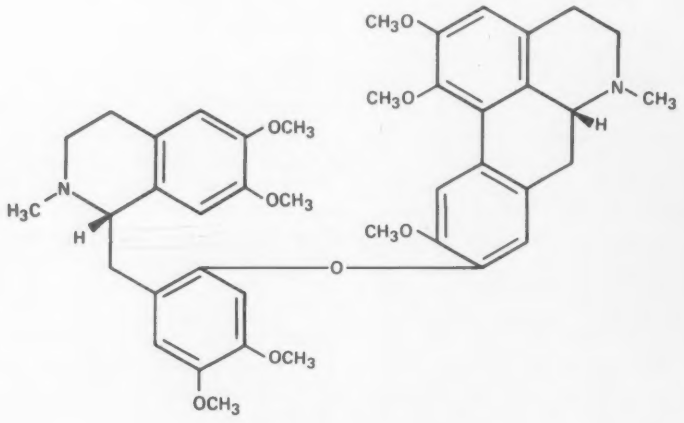


Bruceantin

FIGURE 4

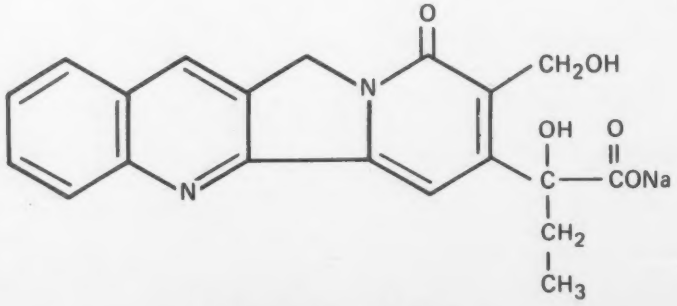
cate any significant degree of binding of thalicarpine to calf thymus DNA (51). In subsequent studies using equilibrium dialysis, however, Allen and Creaven (52) found that tritiated thalicarpine binds to DNA, and that this binding is completely reversible. They also reported that although thalicarpine did not bind to human serum albumin in vitro, it did bind to some unidentified human serum component in vivo (52); it also inhibits aniline hydroxylase activity of rat liver microsomes (53).

On the basis of its activity against the Walker 256 carcinosarcoma, thalicarpine was entered into initial clinical trials, but soon was found to possess serious toxicity at doses below those required for antitumor activity. Although no indication of myelosuppression was noted, serious central nervous system and/or cardiovascular toxicity was encountered in 15 of 16 patients receiving doses ranging between 1070 and 1800 mg/m² (54). Creaven et al examined the physiologic disposition of tritiated thalicarpine in patients with advanced cancer. They found prolonged body retention of thalicarpine with slow and erratic excretion of radioactivity in the urine; maximum urine recovery was about 50% in 65 days. During the first 6 days after treatment, approximately 60% of the urinary radioactivity was attributable to unchanged thalicarpine; fecal recovery was negligible, and no unchanged thalicarpine was found in cerebrospinal fluid (55). The data indicate extensive localization of thalicarpine in tissues and the probability of dangerous drug accumulation if it is given at short dosage intervals.



Thalicarpine

FIGURE 5



Camptothecin sodium

FIGURE 6

Alkaloid of *Camptotheca acuminata* Decne.: Camptothecin

Camptothecin is an alkaloid derived from the tree *Camptotheca acuminata*, indigenous to mainland China. After finding that extracts from this tree had potent antitumor activity in rodents, a search for the active principle in such extracts led to the isolation and structural elucidation of camptothecin by Wall et al (56). This compound is a complex quinoline alkaloid whose structure, as the sodium salt, is shown in figure 6. It was fortunate that the sodium salt of camptothecin was also active in animal tumor systems since the salt is water soluble. Most of the animal and clinical studies described below have been performed with the sodium salt. Structure-activity studies have revealed that although substitutions on the benzene ring do not alter antitumor effects, the lactone group and the 20-hydroxyl group both appear essential for activity. Oxidation of the quinoline to the *N*-oxide reduces, but does not entirely abolish, activity (57).

Camptothecin has antitumor activity in several experimental systems including L1210, P388, L5178Y, and K1964 leukemias. Moreover, it is effective against variants of these tumors resistant to the folic acid antagonists, BCNU, cytosine arabinoside, 6-mercaptopurine, L-asparaginase, and vincristine (58). Camptothecin is also active against the Walker 256 carcinosarcoma in vivo and is cytotoxic in vitro against various leukemia cell lines and PHA-stimulated human lymphocytes (56,58,59). In general, camptothecin is more active against various animal tumors when given at doses of 40 mg/kg every 4 days than when given on a daily basis (58).

Camptothecin is a potent inhibitor of nucleic acid synthesis in HeLa and L1210 cells, and in PHA-stimulated human lymphocytes (58,60,61). DNA and RNA synthesis is inhibited in both HeLa and L1210 cells, although the effect on RNA synthesis is reversible. In addition, exposure of L1210 cells to camptothecin results in degradation of their DNA. The inhibition of nucleic acid synthesis by camptothecin differs in several respects from that of other established inhibitors, and its precise mechanism of action remains unclear.

Studies on the disposition of camptothecin have been performed in rodents and in man (62–64). In mice given a single ip injection, the drug is rapidly cleared from plasma and from kidney and liver tissue, with a half-life of 30 minutes. However, gastrointestinal tissue levels decline more slowly (half-life of 210 minutes), possibly due to enterohepatic circulation of the drug. In fact, biliary transport studies in rats have shown that camptothecin is actively transported into bile and that bile is a major route for its excretion. Camptothecin is extensively bound to plasma proteins in man and in mice with 98% and 70%, respectively, carried in bound form. The α-globulin fraction of purified bovine serum protein demonstrates the highest degree of binding. Plasma levels in man after a single iv dose show a biexponential decay curve; there is no evidence as yet that camptothecin is extensively metabolized in man (64).

The results of phase II studies in 61 patients with advanced gastrointestinal adenocarcinoma (65) and in 15 patients with advanced melanoma (66) showed that camptothecin is without significant therapeutic activity in these neoplasms. However, numerous toxic effects were noted, including myelosuppression, nausea and vomiting, diarrhea, alopecia, and hemorrhagic cystitis (65,66). Thus, the early enthusiasm for the use of camptothecin (67) has been tempered by the frequency and severity of its adverse effects and apparent inactivity in clinical trials.

Quinone of *Stereospermum suaveolens* (Roxb.) DC.: Lapachol

The structures of lapachol and two of its known metabolites, lomatiol and metabolite A, are shown in figure 7. Lapachol was identified as the active principle in extracts of the roots of *Stereospermum suaveolens* which showed antitumor activity against the rat Walker 256 carcinosarcoma (68). Lapachol is active against the im Walker 256 carcinosarcoma when it is given either by the sc or oral route. When given orally, a single dose or multiple doses on alternate days were not as effective as dosing twice daily on Days 3–6 after tumor inoculation. Lapachol did not have activity against various other experimental tumors, including adenocarcinoma 755, L1210 leukemia, or P1534 leukemia; however, the sodium salt of lapachol was active against L1210 leukemia, perhaps because higher blood levels of the salt were attained in comparison to lapachol itself.

The structure-activity relationships of lapachol and various analogs have recently been reviewed (69). Removal of the hydroxyl group or the isopentenyl group reduced Walker 256 antitumor activity by 50%, while removal of the aromatic ring gave an inactive product. Replacement of one or both methyl groups on the side chain by hydrogen abolished activity, although replacement with bromine or chlorine produced compounds as active as lapachol. Reduction, oxidation, or isomerization of the side chain also abolished activity, as did the addition of water to the double bond or the replacement of the hydroxyl by an amino or a methylamino group (69).

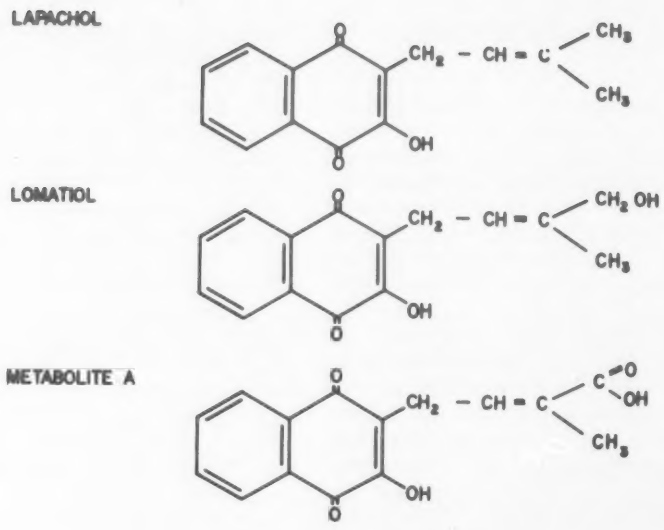


FIGURE 7

Clinical studies have shown that lapachol is well tolerated but that it is without antitumor activity. However, it was probably ineffective in man because effective drug concentrations in blood were never achieved. Thus, although the optimal plasma concentration for activity against the Walker 256 carcinosarcoma has been shown to be 30–35 μg/ml, this plasma concentration was never attained in cancer patients receiving lapachol. Figure 8 shows the levels of unchanged drug in the plasma of a patient receiving 0.5 g of lapachol orally every 6 hours for 5 days. When total quinones in plasma were assayed (including the inactive metabolites lomatiol and metabolite A) a higher but still noncytotoxic level of compounds was detected (70). Similarly, the optimal antitumor concentration of drug in plasma was never achieved in two patients receiving a single oral dose (2 g) of lapachol (fig 9).

At high oral doses, the most important toxic effects of lapachol in man are nausea, vomiting, and a reversible prolongation of the prothrombin time. The latter effect may derive from the structural similarity of lapachol to vitamin K. The clinical studies with lapachol were terminated not only because of its anticoagulant effects, but also because plasma levels of ≥ 30 μg/ml of lapachol could not be achieved without encountering toxicity. A more readily absorbed lapachol derivative (dichloroallyllawsone) may be more useful as a cancer chemotherapeutic agent and is presently under investigation.

LIST OF COMPOUNDS

Bruceantin: NSC-165563
Camptothecin: NSC-100880; CAS reg. No. 25387-67-1
Lapachol: NSC-11905; CAS reg. No. 84–79–7; 1,4-naphthoquinone, 2-hydroxy-3-(3-methyl-2-butenyl)-
Maytansine: NSC-153858
Thalicarpine: NSC-68075; CAS reg. No. 5373–42–2
Vinblastine: NSC-49842; CAS reg. No. 6449–03–2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)
VM-26: NSC-122819; 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside)
VP-16-213: NSC-141540; 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-ethylidene-β-D-glucopyranoside)

REFERENCES

1. GERAN RI, GREENBERG NH, MACDONALD MM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). Cancer Chemother Rep Part 3, vol 3(2):1–103, 1972.
2. SKIPPER HE. The effects of chemotherapy on the kinetics of leukemic cell behavior. Cancer Res 25:1544–1550, 1965.
3. CULPEPER, *in* The Complete Herbal (1653), as cited by Creasey WA, in ref 7.
4. NOBLE RL, BEER CT, and CUTTS JH. Further biological activities of vincaleukoblastine—an alkaloid isolated from *Vinca rosea* (L). Biochem Pharmacol 1:347–348, 1959.
5. JOHNSON IS, WRIGHT HF, and SVOBODA GH. Experimental basis for clinical evaluation of antitumor principles derived from *Vinca rosea* Linn. (Abstr) J Lab Clin Med 54:830, 1959.
6. NEUSS N, GORMAN M, and JOHNSON IS. Natural products in cancer chemotherapy. *In* Methods in Cancer Research (Busch H, ed). New York, Academic Press, 1967, pp 633–702.

7. CREASEY WA. Vinca alkaloids and colchicine. *In* Antineoplastic and Immunosuppressive Agents (Sartorelli AC, and Johns DG, eds). New York, Springer-Verlag, 1975, vol 2, pp 670–694.
8. JOHNSON IS, HARGROVE WW, HARRIS PN, ET AL. Preclinical studies with vinglycinate, one of a series of chemically derived analogs of vinblastine. Cancer Res 26:2431–2436, 1966.
9. JOHNSON IS, ARMSTRONG JG, GORMAN M, ET AL. The vinca alkaloids: a new class of oncolytic agents. Cancer Res 23:1390–1427, 1963.
10. ADAMSON RH, DIXON RL, BEN M, ET AL. Some pharmacologic properties of vincristine. Arch Int Pharmacodyn Ther 157:299–311, 1965.
11. JOHNSON IS. Plant alkaloids. *In* Cancer Medicine (Holland JF, and Frei E, eds). Philadelphia, Lea and Febiger, 1973, pp 840–850.
12. DIXON GJ, DULMADGE EA, MULLIGAN LT, ET AL. Cell culture bioassay for vincristine sulfate in sera from mice, rats, dogs, and monkeys. Cancer Res 29:1810–1813, 1969.
13. MORASCA L, RAINISIO C, and MASERA G. Duration of cytotoxic activity of vincristine in the blood of leukemic children. Eur J Cancer 5:79–80, 1969.
14. BLEYER WA, FRISBY SA, and OLIVERIO VT. Uptake and binding of vincristine by murine leukemia cells. Biochem Pharmacol 24:633–639, 1975.
15. CREASEY WA, SCOTT AI, WEI C-C, ET AL. Pharmacological studies with vinblastine in the dog. Cancer Res 35:1116–1120, 1975.
16. OWELLEN RJ, and HARTKE CA. The pharmacokinetics of 4-acetyl tritium vinblastine in two patients. Cancer Res 35:975–980, 1975.

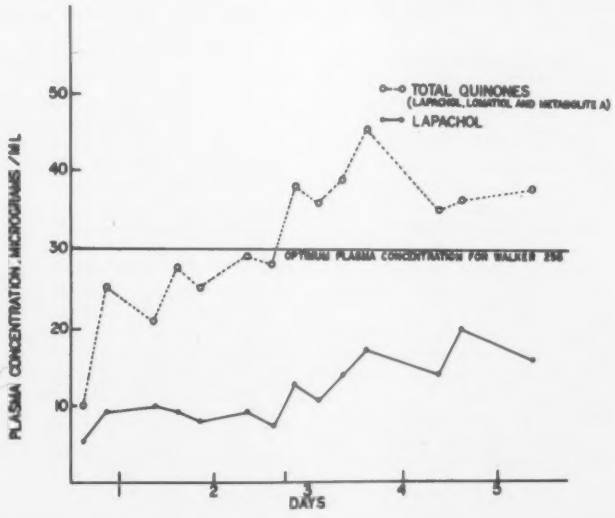


FIGURE 8.—Plasma concentration of lapachol and its quinone metabolites after administration of 0.5 g every 6 hrs for 5 days to a single patient.

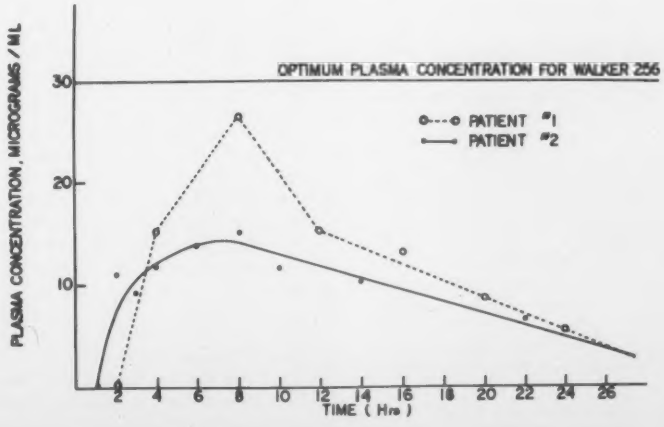


FIGURE 9.—Plasma concentration of lapachol in 2 patients after a single oral dose of 2.0 g.

17. OWELLEN RJ, DONIGIAN DW, HARTKE CA, ET AL. The binding of vinblastine to tubulin and to particulate fractions of mammalian brain. Cancer Res 34:3180–3186, 1974.
18. BENSCH KG, and MALAWISTA SE. Microtubular crystals in mammalian cells. J Cell Biol 40:95–107, 1969.
19. BENTLEY R. New American remedies. I: *Podophyllum peltatum*. Pharm J Trans 3:456–464, 1861.
20. KAPLAN IW. Condyloma acuminata. N Orleans Med Surg J 94:388–390, 1942.
21. KELLER-JUSLÉN C, KUHN M, VON WARTBURG A, ET AL. Synthesis and antimitotic activity of glycosidic lignan derivatives related to podophyllotoxin. J Med Chem 14:936–940, 1971
22. STÄHELIN H. 4'-Demethyl-epipodophyllotoxin thenylidene glucoside (VM 26), a podophyllum compound with a new mechanism of action. Eur J Cancer 6:303–311, 1970.
23. STÄHELIN H. Activity of a new glycoside lignan derivative (VP 16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer 9:215–221, 1973.
24. AVERY TL, ROBERTS D, and PRICE RA. Delayed toxicity of 4'-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) in mice. Cancer Chemother Rep 57:165–173, 1973.
25. DOMBERNOWSKY P, NISSEN NI, and LARSEN V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4'-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
26. NISSEN NI, LARSEN V, PEDERSEN H, ET AL. Phase I clinical trial of a new antitumor agent, 4'-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 56:769–777, 1972.
27. SONNTAG RW, SENN HJ, NAGEL G, ET AL. Experience with 4'-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM-26; NSC-122819 in the treatment of malignant lymphosis. Eur J Cancer 10:93–98, 1974.
28. MATHÉ G, SCHWARZENBERG L, POUILLART P, ET AL. Two epipodophyllotoxin derivatives, VM 26 and VP 16213, in the treatment of leukemias, hematosarcomas, and lymphomas. Cancer 34:985–992, 1974.
29. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, JR, ET AL. 4'-Demethyl-epipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.
30. ALLEN LM, and CREAVEN PJ. Comparison of the human pharmacokinetics of VM-26 and VP-16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11:697–707, 1975.
31. CREAVEN PJ, and ALLEN LM. Clinical pharmacology of the antineoplastic epipodophyllotoxin VM-26. In press.
32. HUANG CC, HOU Y, and WANG JJ. Effects of a new antitumor agent, epipodophyllotoxin, on growth and chromosomes in human hematopoietic cell lines. Cancer Res 33:3123–3129, 1973.
33. MISRA NC, and ROBERTS DW. Inhibition by 4'-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) of human lymphoblast cultures in G_2 phase of the cell cycle. Cancer Res 35:99–105, 1975.
34. GOSALVEZ M, PEREZ-GARCIA J, and LOPEZ M. Letter: Inhibition of NADH-linked respiration with the anti-cancer agent 4'-demethyl-epipodophyllotoxin thenylidene glucoside (VM-26). Eur J Cancer 8:471–473, 1972.
35. KUPCHAN SM, KOMODA Y, COURT WA, ET AL. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J Am Chem Soc 94:1354–1356, 1972.
36. KUPCHAN SM, KOMODA Y, BRANFMAN AR, ET AL. Novel maytansinoids. Structural interrelations and requirements for antileukemic activity. J Am Chem Soc 96:3706–3708, 1974.
37. WOLPERT-DEFILIPPES MK, ADAMSON RH, CYSYK RL, ET AL. Initial studies on the cytotoxic action of maytansine, a novel ansa macrolide. Biochem Pharmacol 24:751–754, 1975.
38. ADAMSON RH, WOLPERT MK, SIEBER SM, ET AL. Experimental studies with maytansine—a new antileukemic agent. (Abstr) *In* Proceedings of the 7th International Symposium on Comparative Leukemia Research, Copenhagen, Oct 13–17, 1975, p 148.
39. WOLPERT-DEFILIPPES MK, BONO VH, JR, DION RL, ET AL. Initial studies on maytansine-induced metaphase arrest in L1210 murine leukemia cells. Biochem Pharmacol 24:1735–1738, 1975.
40. REMILLARD S, REBHUN LI, HOWIE GA, ET AL. Antimitotic activity of the potent tumor inhibitor maytansine. Science 189:1002–1005, 1975.
41. HARTWELL JL. Plants used against cancer. A survey. Lloydia 34:204–255, 1971.
42. KUPCHAN SM, BRITTON RW, ZIEGLER MF, ET AL. Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J Org Chem 38:178–179, 1973.
43. KUPCHAN SM, BRITTON RW, LACADIE JA, ET AL. The isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from *Brucea antidysenterica*. J Org Chem 40:648–654, 1975.
44. LIAO L-L, HORWITZ SB, and GROLLMAN AP. Bruceantin: an inhibitor of protein biosynthesis. (Abstr) Fed Proc 33:581, 1974.
45. LIAO L-L, KUPCHAN SM, and HORWITZ SB. Mode of action of the antitumor compound bruceantin, an inhibitor of protein synthesis. Mol Pharmacol 12:167–176, 1976.
46. KUPCHAN SM, CHAKRAVARTI K, and YOKOYAMA N. Thalicarpine, a new hypotensive alkaloid from thalictrum dasycarpum. J Pharm Sci 52:985–988, 1963.
47. KUPCHAN SM. Recent advances in the chemistry of tumor inhibitors of plant origin. Trans NY Acad Sci 32:85–106, 1970.
48. MOLLOV NM, DUTSCHEWSKA HB, SILJANOVSKA K, ET AL. Cytotoxic effect of alkaloids from *Thalictrum* minus ssp. elatum and their derivatives. Dokl Bolg Akad Nauk 21:605–608, 1968.
49. HAHN RA, NELSON JW, TYE A, ET AL. Pharmacological activity of thalicarpine. J Pharm Sci 55:466–469, 1966.
50. HERMAN EH, and CHADWICK DP. The cardiovascular actions of thalicarpine. Toxicol Appl Pharmacol 26:137–147, 1973.
51. ALLEN LM, and CREAVEN PJ. Inhibition of macromolecular biosynthesis in cultured L1210 mouse leukemia cells by thalicarpine (NSC 68075). Cancer Res 33:3112–3116, 1973.
52. ALLEN LM, and CREAVEN PJ. Binding of a new antitumor agent, thalicarpine, to DNA. J Pharm Sci 63:474–475, 1974.
53. CREAVEN PJ, ALLEN LM, and WILLIAMS CP. The interaction of the antineoplastic drug thalicarpine with aniline hydroxylase and microsomal cytochrome of rat liver. Xenobiotica 4:255–261, 1974.
54. CREAVEN PJ, COHEN MH, SELAWRY OS, ET AL. Initial clinical trial of thalicarpine, an antineoplastic plant alkaloid of novel structure. (Abstr) *In* Proceedings of the 9th International Congress of Chemotherapy, London, 1975.
55. CREAVEN PJ, ALLEN LM, and WILLIAMS CP. Clinical pharmacology of thalicarpine, a new antineoplastic plant alkaloid. (Abstr) *In* Proceedings of the 6th International Congress of Pharmacology, Helsinki, Finland, July 20–26, 1975.
56. WALL ME, WANI MC, COOK CE, ET AL. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890, 1966.
58. GALLO RC, WHANG-PENG J, and ADAMSON RH. Studies on the antitumor activity, mechanism of action, and cell cycle effects of camptothecin. J Natl Cancer Inst 46:789–795, 1971.
59. VENDITTI JM, and ABBOTT BJ. Studies on oncolytic agents

from natural sources. Correlations of activity against animal tumors and clinical effectiveness. Lloydia 30:332–348, 1967.
60. HORWITZ SB, CHANG C, and GROLLMAN AP. Mechanism of action of camptothecin. Pharmacologist 12:1283, 1970.
61. KESSEL D. Effects of camptothecin on RNA synthesis in leukemia L1210 cells. Biochim Biophys Acta 246:225–232, 1971.
62. HART LG, CALL JB, and OLIVERIO VT. A fluorometric method for determination of camptothecin in plasma and urine. Cancer Chemother Rep 53:211–214, 1969.
63. GUARINO AM, ANDERSON JB, STARKWEATHER DK, ET AL. Pharmacologic studies of camptothecin (NSC-100880): distribution, plasma protein binding, and biliary excretion. Cancer Chemother Rep 57:125–140, 1973.
64. CREAVEN PJ, and ALLEN LM. Renal clearance of camptothecin (NSC-100880): effect of urine volume. Cancer Chemother Rep 57:175–184, 1973.
65. MOERTEL CG, SCHUTT AJ, REITEMEIER RJ, ET AL. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 56:95–101, 1972.
66. GOTTLIEB JA, and LUCE JK. Treatment of malignant melanoma with camptothecin (NSC-100880). Cancer Chemother Rep 56:103–105, 1972.
67. GOTTLIEB JA, GUARINO AM, CALL JB, ET AL. Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). Cancer Chemother Rep 54:461–470, 1970.
68. RAO KV, MCBRIDE TJ, and OLESON JJ. Recognition and evaluation of lapachol as an antitumor agent. Cancer Res 28:1952–1954, 1968.
69. DRISCOLL JS, HAZARD GF, JR, WOOD HB, JR, ET AL. Structure-antitumor activity relationships among quinone derivatives. Cancer Chemother Rep Part 2, vol 4(2):1–362, 1974.
70. BLOCK JB, SERPICK AA, MILLER W, ET AL. Early clinical studies with lapachol (NSC-11905). Cancer Chemother Rep Part 2, vol 4(4):27–28, 1974.

Plant Products in Cancer Chemotherapy

Stephen K. Carter[1,2,*] and Robert B. Livingston[3]

Cancer chemotherapy is a relatively new specialty in medical oncology which began soon after the end of World War II, stimulated by the discovery of the clinical activity of mechlorethamine in 1943. Today, there are many drugs with a wide range of chemical structures having some clinical activity against one or more kinds of human malignancy.

Before a new drug is ready for clinical evaluation, it undergoes extensive toxicologic and pharmacologic evaluations in two large animal species, the beagle hound and rhesus monkey, to define a series of dose levels from nontoxic to lethal. The dose of prime interest to the clinician is the toxic dose-low (TDL), which is the first dose level measured in mg/m^2 of body surface that produces a degree of toxicity. Dose-response characteristics are also determined, for if the toxicity curve of a drug is steep, more caution will have to be used as dose levels are increased during the initial clinical evaluation. Data are also obtained on major organ toxicity in terms of parameters and whether such toxicity is predictable, manageable, and reversible. The preclinical studies also provide information concerning response to different schedules and routes of administration.

Patients selected for phase I clinical trials have advanced disease which is refractory to the standard therapy for their particular neoplasm. They are usually patients who have been treated with surgery, radiotherapy, and/or chemotherapy that could be of benefit to them. Their disease need not be measurable or evaluable since the principal goals of the phase I clinical evaluation are to determine the maximum tolerated dose (MTD) at one or more schedules, to establish the parameters of toxicity (ie, damage to vital organs), and to determine if the toxicity is usual, predictable, treatable, and/or reversible. If the toxicity is not unusual or unpredictable then the drug receives consideration for the phase II trial. The usual toxic effects occur in the bone marrow, gastrointestinal (GI) tract, liver, and kidneys; these can be predicted and managed.

It should be emphasized that phase I studies are therapeutic trials and beneficial patient responses do occur in some instances. Although such antitumor effects are encouraging when observed, they are not essential in order for a drug to proceed to phase II evaluation. Since most phase I patients do not have measurable lesions, it is sometimes difficult to establish whether a drug is clinically active or inactive. On the other hand, there are many examples of phase I patients who have been subjected to every standard treatment and have responded dramatically to phase I drugs.

The phase I evaluation is conducted with 30–50 patients. The initial dose used is one third of the TDL (mg/m^2) established in the large animal toxicology studies. The new drug is initially administered at this low dose and then increased until the MTD is reached. The first escalation may be 100%, the second 75%, the third and fourth 50% and 33%, and so forth; that is, as the toxic dose is approached, the dose escalation gets smaller.

Phase II clinical evaluation encompasses the screening of new drugs for clinical activity. Fifteen to 30 evaluable patients are treated who have measurable indicator lesions in each of a range of representative or signal tumor types and the objective response is evaluated in each. Drugs are tested against fast-growing tumors (eg, leukemia and lymphoma) and slow-growing tumors (eg, colon, breast, and lung). The term screen is appropriate for it is impossible to test each new drug in every kind of human cancer where chemotherapy might be of value. If the drug is not active against any of the signal tumors, it is unlikely to be of value in chemotherapy.

Results from the phase II evaluation are used to determine whether a new drug will be taken into phase III trials. A new compound which is active against one of the signal tumor types will not be of great interest for phase III unless its activity equals or surpasses that of previously established drugs. For example, a number of compounds can produce response rates of 50%–80% in Hodgkin's disease and a new drug with only 15%–20% activity

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s likely to be of little interest for phase III trials in hat disease. On the other hand, in cancer of the ɔancreas, colon, or lung where the best drugs are ıctive in the range of 20%, a compound with only 15%–20% activity might be worthy of further study. Toxicity of the compound is also a factor in the decision to proceed to phase III. A compound with ɔnly a 30% response rate in Hodgkin's disease could be of interest if it lacks bone marrow toxicity because this toxicity is characteristic of the compounds now available for treating this disease.

In addition, the pharmacologic and biochemical characteristics of a drug are factors in making the decision to proceed to phase III. Selection of a drug for phase III is enhanced if it is a new chemical structure which is cell-cycle nonspecific and exhibits the ability to kill nonproliferating cells or attack cancer in pharmacologic sanctuaries like the central nervous system. If a compound seems suitable for combination chemotherapy (eg, a lack of bone marrow toxicity), its chances of reaching phase III are increased because it might be combined with other drugs that do have this toxicity.

At the phase III stage of clinical evaluation new drugs are tested in controlled clinical trials and combination studies to determine their role in cancer therapy. The classic trial is the comparison of a new active drug with the standard treatment used in a particular disease. If, for example, the drug was active against colon cancer in phase II studies, the classic phase III evaluation would be a controlled comparison to 5-fluorouracil (5-FU), the standard drug treatment for that disease, to determine which was superior.

Increasingly, the phase III trials are moving away from such classic studies and into combination chemotherapy for the major solid tumors. In this approach, new drugs will be tested in combination with existing drugs against advanced disease to develop optimal chemotherapy regimens for primary and disseminated disease. Attempts will then be made to integrate the optimal chemotherapy regimen into a combined modality approach to the primary treatment of local and regional disease. There is now ample evidence showing that surgery often removes the bulk of the cancer but leaves behind microscopic metastatic foci of tumor cells that later grow and become large bulky lesions which eventually kill the patient. We feel that chemotherapy should be used immediately after the local modalities of surgery and radiotherapy to eradicate these foci and increase the cure rate.

In research with cancer patients, the welfare of the patient is always of prime concern to the clinician. The term control is not applicable here in the usual sense. Patients are not separated into test and control groups. Instead, some patients receive a standard chemotherapy regarded as most suitable for their disease, in contrast to others who receive an experimental therapy which hopefully will be superior to the standard therapy. If either therapy fails in these controlled trials, there can be a crossover and the patient will be treated with the other drug. In these crossover clinical trials the only question is—which patients will first receive the standard therapy and which will first receive the experimental therapy?

Natural products have been an active part of the clinician's resources since the early days of cancer chemotherapy (1–5). As far back as 1947, Belkin (1) and Hartwell and Shear (2) reported the destructive effects of podophyllin on experimental mouse tumors and the older literature contains scattered references to the use of these substances in folk medicine. It was used as a cancer remedy by the Penobscot Indians of Maine (3) and by private practitioners treating cancer in the US before 1897 (4). Two new derivatives of podophyllin are presently under active clinical investigation.

Reports of clinical antitumor activity by colchicine appeared as early as 1935 (5) but this drug and its derivatives have not been active enough to have a significant role in cancer chemotherapy.

The plant products that have played the most vital role are the vinca alkaloids. This paper will review the clinical data on the vinca alkaloids, attempting to place them in clinical perspective, and briefly consider the data on investigational plant products currently in clinical trial.

Vinca Alkaloids

The vinca alkaloids evolved from independent investigations in Canada and the US. From 1949 to 1955 Noble, Beer, and Cutts (6,7), at the University of Western Ontario, studied extracts from the periwinkle *Vinca rosea (Catharanthus roseus* Linn.), which is indigenous to the West Indies, to determine if they possessed hypoglycemic activity. Hot aqueous ethanol extracts of the leaves, stem, and root were made and acidified with acetic acid. There was no hypoglycemic action in rats but leukopenia was observed and suggested a potential antileukemic effect (6–8).

Independently, Johnson, Wright, and Svoboda at the Lilly Research Laboratories were conducting a routine screening program on a wide range of potential antitumor agents. In December 1957, they found that a crude extract of the *Vinca rosea* plant had profound antileukemic activity against the P1534 transplantable mouse leukemia (9,10). Subsequently, both the US and Canadian groups began a

series of studies to determine the chemical nature of the active principle and ultimately this led to clinical trials.

Two major drugs derived from the periwinkle, vincristine (VCR) and vinblastine (VBL), have achieved established status. The vinca alkaloids are large and complex molecules (11) which differ structurally only in the methyl (VBL) or formyl (VCR) side-chains on a large parent molecule. Despite their apparently minor chemical differences, the two molecules differ in their antitumor spectra and have widely different clinical toxicities.

Current thinking on the mechanism of action of the vinca alkaloids postulates at least two separate sites of action, ie, reversible mitotic arrest through binding of drug to cytoplasmic precursors of the spindle and inhibition of RNA synthesis through effects on the DNA-dependent RNA polymerase system (12). The binding of the drug to spindle precursors may take place during the S phase, when centriolar replication occurs, with cellular spindle disorganization and consequent death not becoming apparent until the next mitosis is attempted (13). On a molecular level, it has been suggested that the vinca alkaloids cause a rearrangement of binding sites in the protein of the microtubular units which comprise the mitotic spindle, permitting polymerization of the tubule protein to protofibrils (14).

VBL

The standard dose schedule of VBL is iv administration of 0.1–0.15 mg/kg weekly. Depending on tumor response and individual patient tolerance the doses can then be progressively increased to as high as 0.3 mg/kg once weekly. Hematologic toxicity is the usual dose-limiting side effect, with the wbc nadir depression usually occurring 4–7 days after a single injection. Thrombocytopenia is also observed. Nausea, vomiting, and anorexia are common while stomatitis is usually associated with leukopenia. Neurologic complications can occur but are much less common than with VCR.

For the most part VBL is useful only in the treatment of Hodgkin's disease. The overall response rate determined in a literature review (15) is 68% with 463 responses in 682 evaluable patients (table 1). More significantly, the CR rate of 30% (89 of 293 patients) is more than twice that reported for alkylating agents.

In a study reported by Carbone et al (16) of the Acute Leukemia Group B (ALGB) cyclophosphamide (15 mg/kg) given iv each week was compared to VBL (0.1–0.3 mg/kg) given iv weekly for remission induction of Hodgkin's disease. In the previously untreated group the CR rate with VBL was 27% (15 of 56 patients) versus 18% (ten of 54 patients) for cyclophosphamide. The total response rate for VBL was significantly better than that for cyclophosphamide ($P < 0.05$), ie, 75% (42 of 56 patients) versus 54% (29 of 54 patients). In previously treated patients the two drugs gave essentially the same results. It was believed that the sequence of VBL followed by cyclophosphamide was superior to the reverse order.

In a similar comparative study reported by Stutzman et al (17) VBL produced 11 CRs in 33 patients with a total response rate of 85% versus only two CRs in 14 patients with a total response rate of 43% for cyclophosphamide, the latter given orally at 2 mg/kg/day for 6 weeks. In another study, however, Ezdinli and Stutzman (18) compared VBL with mechlorethamine and obtained results that were the same for induction with either agent, although the sequence of mechlorethamine followed by VBL gave an 89% response rate versus 65% for the reverse order. These data indicate again, in an indirect manner, the superiority of mechlorethamine over cyclophosphamide for treatment of this disease. When directly compared, cyclophosphamide appears to be distinctly inferior to VBL, but the superiority disappears when mechlorethamine is used as the alkylating agent in the comparison with VBL.

Despite this activity, VBL has not played a major role in combination approaches because of the superiority of VCR for combination usage.

VBL has activity against the non-Hodgkin's lymphomas with an overall response rate of 27% (24 of 89 patients) in a cumulative data review (36). The CR rate was low (three CRs among 54 patients analyzed) and VCR is thought to be the superior agent and is used in combination-drug regimens.

In early clinical trials VBL demonstrated activity against methotrexate-resistant choriocarcinoma (37). It has been largely superseded by actinomycin D as a second-line drug after methotrexate in this disease.

VBL has undergone fairly extensive trial in the major solid tumors. The overall response rate in breast cancer is 20% (38), but marked variability exists among the results obtained by different investigators (28,39–41). In a combined total of 54 malignant melanoma patients reported, there have been eight responses for a response rate of 15% (36). Against bronchogenic and colon cancer VBL has shown only slight activity (36).

VBL has activity against testicular carcinoma. Samuels and Howe (42) used the drug quite intensively (0.4–0.8 mg/kg/week) and reported four CRs and seven PRs among a variety of cell types in 21

TABLE 1.—VBL therapy for Hodgkin's disease

Investigator(s)	Ref No.	Dose schedule	No. of evaluable patients	Overall response*	
				No. of responses	%
Warwick et al	19	0.2 mg/kg in 10 divided doses or 0.02 mg/kg iv at hourly intervals	27	16	59
Whitelaw and Teasdale	20	10 mg/q3–4days iv	15	13	87
Hodes et al	21	0.15–0.2 mg/kg/wk iv	14	9	64
Armstrong et al	22	0.1–0.3 mg/kg/wk iv	10	10	100
Frost et al	23	0.3–0.6 mg/kg in 3 days or 0.1–0.2 mg/kg weekly or q2wks	22	8	36
Midwest Cooperative Chemotherapy Group	24	0.1–0.15 mg/kg/day × 1–3; then 0.1 mg/kg q4–7days according to severity of leukopenia	21	12	57
Marsden	25	0.15–0.2 mg/kg/wk	14	9	64
Bernard et al	26	0.15–0.3 mg/kg/wk iv	85	55	65
Butler	27	0.15–0.2 mg/kg/wk iv	11	6	54
ECOG-ALGB	28	0.1→0.3 mg/kg/wk iv	38	31	82
Bond et al	29	0.09 mg/kg/day orally	12	5	42
Stutzman et al	17	0.15–0.2 mg/kg/wk × 6 iv	33	28	85
MacDonald and Lacher	30	0.19–0.85 mg/wk orally	12	8	67
Viala et al	31	5→20 mg/wk iv	50	30	60
Ezdinli and Stutzman	18	0.15–0.2 mg/kg/wk iv	27	20	74
Sohier et al	32	0.05–0.2 mg/kg/wk iv	35	22	63
Carbone et al	16	0.1→0.3 mg/kg/wk iv	88	57	65
Anglesio et al	33	10 mg/wk iv	23	18	78
Flatow et al	34	0.15→0.25 mg/kg weekly or biweekly iv	19	13	68
Jelliffe	35	10→20 mg/wk iv	126	93	74
Totals			682	463	68

*Data for CR rate available in only 293 of a total of 682 patients; there were 89 CRs among 293 patients (30%).

patients. Smart et al (43) administered a more standard dosage and obtained two PRs.

Samuels et al (44) at the M. D. Anderson Hospital were the first to combine bleomycin with VBL in testicular tumor therapy. Bleomycin (15 mg im) was administered twice weekly for 5 weeks and VBL (0.4–0.6 mg/kg iv) was given in two fractions (Days 1 and 2). Fifty patients were treated by this induction scheme and, if response was seen, additional therapy consisted of three or four courses of the same VBL dose and 50% of the bleomycin dose. Sixteen patients achieved CR (32%) with this regimen and 15 were disease free after 2 years. Twenty-two other patients experienced a PR (> 50% reduction in maximum tumor diameter) with a median survival of 32 weeks. Five patients developed interstitial pneumonitis secondary to bleomycin therapy.

Samuels (45) has modified his approach to a regimen in which VBL (0.4 mg/kg) is given in two fractions (Days 1 and 2) and bleomycin (30 mg in 1000 ml of 5% dextrose in water over 24 hours) is started on Day 2 for 5 additional days. Courses are repeated every 21–28 days × 3–4. Forty stage III germinal tumors and four extragonadal primary tumors have been studied. In 39 evaluable patients with high tumor volume, there were 19 CRs (47%) and ten PRs. In the extragonadal group one CR and PR each were seen. The mean survival of complete responders is 34 weeks with no deaths. Toxicity included severe leukopenia in 40 patients, thrombocytopenia in 21, hemolytic anemia in 13, stomatitis in all, and bleomycin pneumonitis in two. It appears that the CR rate is superior to that produced by intermittent bleomycin and VBL therapy.

At the Memorial Sloan-Kettering Cancer Center, New York, NY, daily doses of a three-drug combination of bleomycin (0.4 mg/kg), actinomycin D (0.0075–0.015 mg/kg), and VBL (0.025–0.05 mg/kg) have been used in testicular cancer (46). Each drug was given iv on Days 1–3 and repeated for two to three doses in 7–14 days as toxicity permitted. Twenty-one patients were treated and 16 were evaluable; eight of

these showed objective responses lasting 1–5 months. Hematologic toxicity was predictable and mucocutaneous toxicity between Days 4 and 20 was frequently severe and dose-limiting. Pulmonary toxicity was observed in one patient.

VCR

The usual dosage of VCR is 1.5–2.0 mg/m² iv. It is recommended that individual doses in adult patients should not exceed 2 mg and older adults may require considerable dose reduction to avoid severe neurotoxicity.

Neurotoxicity is the most common side effect of VCR, but there is no general agreement as to the exact site of the initial neural lesion. Different investigators have suggested sensory cells of dorsal root ganglia and anterior horn cells (47), dorsal root cells (48), muscle spindle system (49), and the axon (50) as the site affected.

The earliest and most consistent objective manifestation of VCR neurotoxicity is suppression of the Achilles tendon reflex (51). Loss of the Achilles reflex is asymptomatic in 60% of the patients with 48% eventually manifesting depression of other deep tendon reflexes, including complete areflexia (52).

Paresthesia involving the feet or hands (or both) is an early subjective manifestation of VCR neuropathy, often occurring within the first few weeks of therapy (53). If the drug is stopped at the onset of paresthesia the symptoms disappear, although in some patients this may take several months. Serious objective sensory loss is relatively uncommon.

Motor involvement is one of the most disabling manifestations of VCR neurotoxicity. Characteristically, the weakness impairs the dorsiflexors of the toes and ankles and the extensors of the fingers and wrists (53,54). The loss of deep tendon reflexes almost invariably precedes the onset of objective weakness.

The earliest symptoms of VCR toxicity are often colicky abdominal pain and constipation. These complaints are common and occurred in one third of a large series of patients studied by Holland et al (55). The acute GI symptoms appear within a few days of VCR administration and precede the earliest signs of peripheral neuropathy, such as reflex depression, by several days. The constipation is sometimes troublesome and may cause fecal impaction high in the colon. Other manifestations of autonomic dysfunction that do not occur as frequently as the GI disturbances but may be equally difficult include bladder atony (56), impotence (55), and orthostatic hypotension (57). Alopecia, often total, occurs in almost half the patients but regrowth begins with cessation of the drug. Bone marrow toxicity has been a problem only with high-dose regimens. As with VBL, injection of VCR outside the vein leads to prolonged painful induration of the subcutaneous tissue.

VCR is a highly active agent in the treatment of childhood leukemia. It is now rarely used alone but earlier studies as a single agent were extensive with a total CR rate of 41% in 349 children with acute lymphoblastic leukemia (ALL) (36). In a typical experience reported by the ALGB in 1966 (58) there were 64 CRs among 117 patients given the drug for remission induction. Within the subgroup of ALL the CR plus PR rate was 73% in 103 patients treated. VCR is a mainstay of all the highly effective, complicated combination regimens which are giving impressive long-term survival rates in children with leukemia and have been recently reviewed (58,59).

VCR is also of significant value in the therapy of childhood solid tumors, especially Wilms' tumor. Sullivan (60) reported eight CRs using the drug in 12 children with this disease. The cumulative data show a 74% response rate in 31 patients (36) and VCR is an active component of the current Wilms' Tumor Study Group protocols in which chemotherapy, surgery, and radiotherapy are combined to achieve a high percentage of long-term survivors.

VCR used alone produces an objective response in a significant number of neuroblastoma patients but does not produce a cure (36). A combination of VCR and cyclophosphamide has apparently cured a few children with neuroblastoma even when they have marrow and bone involvement (61). VCR has also proved useful in the treatment of embryonal rhabdomyosarcoma (62–64).

VCR has not been as intensively studied as VBL in Hodgkin's disease. As seen in table 2, only 115 patients are reported for single-agent use and the response rate is 58%. The CR rate is an impressive 36% but unfortunately the numbers are quite small (ten of 28 patients).

The first success with a multiple-drug combination against Hodgkin's disease was achieved by Lacher and Durant (72) at the Memorial Hospital using VBL and chlorambucil. The CR rate was 62% (ten of 16 patients) and the overall response rate was 81%, with a median duration of response of 2–12+ months. The patients had stage II and III disease and all had been previously treated with radiotherapy. While chlorambucil and VBL have overlapping hematologic toxicity, the combination was well-tolerated and the CR rate exceeded that previously observed with either agent alone. In spite of the small number of patients treated and the fact that it was an uncontrolled study, this

TABLE 2.—VCR therapy for Hodgkin's disease

Investigator(s)	Ref No.	Dose schedule	No. of evaluable patients	Overall response*	
				No. of responses	%
Carbone et al	65	0.025–0.075 mg/kg/wk	10	10	100
Whitelaw and Kim	66	2–3 mg twice a week × 2; then weekly	12	5	42
Bohannon et al	67	0.03–0.05 mg/kg/wk	23	9	39
Martin and Compston	68	0.01–0.06 mg/kg/wk	6	5	83
Gailani	69	75 μg/kg/wk	21	10	48
Selawry et al	70	2.5 mg/m²/wk	12	9	75
Desai et al	71	3 mg/wk × 3 or more doses	19	12	63
Carter and Livingston	15†	Weekly iv	12	7	59
Totals			115	67	58

*Data for CR rate available in only 28 of a total of 115 patients; there were 10 CRs among 28 patients (36%).

†Miscellaneous group of studies.

TABLE 3.—MOPP combination therapy of DeVita et al (76)

Drug	Dose schedule	No. of evaluable patients	Response		Overall response rate
			CR	PR	
Mechlorethamine	6 mg/m², Days 1 and 8 iv	43	35 (81%)	6 (14%)	95%
VCR	1.4 mg/m², Days 1 and 8 iv				
Procarbazine	100 mg/m²/day × 14 orally				
Prednisone	40 mg/m²/day × 14 (cycles 1 and 4 only)				
Six 2-week cycles, 14 days on and 14 days off					

initial effort pointed the way to the triumphs of the future.

At about the same time, pioneering work on combination chemotherapy for hematologic malignancies, including lymphoma, was under way at the NCI under the leadership of Zubrod, Frei, and Freireich. The first NCI combination for Hodgkin's disease included cyclophosphamide, VCR, methotrexate, and prednisone given in three 2-week courses and combined with radiotherapy between the first and second course in patients with stage I–IIIA disease (73). Patients with stage IIIB, and some with IIIA, were treated by chemotherapy alone. All nine of the patients who received chemotherapy plus radiotherapy achieved CR and in five patients treated by chemotherapy alone there were three CRs and two PRs. The overall response rate of 100% in this pilot study demonstrated for the first time the feasibility of an aggressive combined modality approach to therapy.

After Frei's departure to the M. D. Anderson Hospital, MOPP (mechlorethamine, VCR, procarbazine, and prednisone), a new combination, was devised and tested at the NCI by DeVita et al (74–78) (table 3). Of the 43 patients who received MOPP therapy in the initial uncontrolled study, 35 achieved CR (81%) and six had PRs for an overall response rate of 95%. The median duration of remission was 36 months and 15 of the CRs were reported disease free for ≤ 7 years (77).

The MOPP combination is the focal point from which all subsequent studies of combination chemotherapy against Hodgkin's disease must be viewed. Since the original MOPP investigation the efforts of various cooperative groups have centered on confirmation of the NCI results and attempts were made to improve the induction rate, duration of disease-free interval, and survival by numerous approaches. These attempts include: (*a*) testing maintenance regimens subsequent to MOPP induction; (*b*) testing

three-drug combinations drawn from available active drugs; (*c*) sequential use of active agents; (*d*) substitution of drugs for those in the MOPP combination (especially nitrosoureas for mechlorethamine and VBL for VCR); (*e*) addition of a fifth drug to MOPP (bleomycin or a nitrosourea); (*f*) new five-drug combinations involving the addition and substitution of drugs (eg, BCVPP [BCNU, cyclophosphamide, VBL, procarbazine, and prednisone]); (*g*) addition of another active modality (radiotherapy) to an active drug combination; and (*h*) development of a new non-cross-resistant combination (eg, ABVD [adriamycin, bleomycin, VBL, and DTIC]). A complete consideration of these studies is outside the scope of this paper but a review has recently been published (79).

The vinca alkaloids, especially VCR, are among the most active drugs in the treatment of non-Hodgkin's lymphomas. A review of the cumulative literature shows a 53% response rate in 93 patients with lymphocytic lymphoma and a 61% response rate in 72 patients with histiocytic lymphoma (36). In 1967 a study using cyclophosphamide, VCR, and prednisone (CVP) in an intensive series of treatment cycles was instituted at the NCI for the treatment of lymphocytic lymphoma (80). Of 46 patients treated, 25 achieved a CR (54%) and 14 had > 75% reduction of tumor volume (PR). A followup of the data with this regimen and with the MOPP regimen has shown that 61% of the patients achieved CR with chemotherapy and that 28% had disease-free survival lasting from 1 to > 7 years after all drug treatment was stopped (81). When the diffuse histiocytic lymphoma group was viewed separately, 11 of 27 patients achieved CR (41%) with a VCR combination (either CVP or MOPP) and ten were disease free when last seen 26–105 months from the end of treatment, indicating that diffuse histiocytic lymphoma is potentially curable (82).

VCR is an essential part of almost every major combination for the non-Hodgkin's lymphomas but a review of this exciting area is outside the scope of this paper.

The vinca alkaloids have induced objective tumor regression in patients with breast cancer. VCR has been the more intensively studied of the two compounds and the data from five major studies plus miscellaneous data of the Cancer Therapy Evaluation Program, DCT, NCI (223 patients) are summarized in table 4. Forty-seven responses have been reported for an overall response rate of 21%. When the two largest series (55,83) are examined in more detail, a dose-response effect for regression is observed. At a dose level of 12.5 μg/kg no responses were obtained in either series but the response rate increases with the dose level until at 75 μg/kg the response rate is 33%. In the study of Holland et al (55) hormone treatments and endocrine ablation had no apparent effect on the frequency of PRs obtained with VCR.

In 1969 at the meeting of the American Association for Cancer Research, Cooper (86) of the Buffalo Medical Group, NY, presented the results of a five-drug combination as follows: 5-FU (12 mg/kg/day × 4, then 500 mg/week iv); methotrexate (25–50 mg/week iv); VCR (0.035 mg/kg/week iv); cyclophosphamide (2.5 mg/kg/day orally); and prednisone (0.75 mg/kg/day orally). Cooper reported a 90% CR rate in 60 hormone-resistant patients with far-advanced breast cancer.

Other investigators have experienced variable success in their attempts to reproduce Cooper's data. A review of the published literature shows nine series (table 5) with response rates ranging from 29% to 70% and an overall response rate of 50% in 348 patients. It must be emphasized that almost every series used some modification of Cooper's original schedule and dose for the five

TABLE 4.—VCR therapy for breast cancer

Investigator(s)	Ref No.	Dose schedule	No. of evaluable patients		Overall response: No. of responses		Overall response: %
Gailani	69	50–75 μg/kg/wk	43		11		26
Grinberg et al	83	12.5 μg/kg/wk	18	75	0	10	13
		25 μg/kg/wk	25		3		
		35 μg/kg/wk	32		7		
Goldenberg	84	0.01–0.05 μg/kg/wk	15		3		20
Silva et al	85	Not stated	10		1		10
Holland et al	55	12.5–75 μg/kg/wk	62		15		24
Carter and Livingston	15*	Weekly	18		7		40
Totals			223		47		21

*Miscellaneous group of studies.

TABLE 5.—Treatment of advanced breast carcinoma by 5-drug chemotherapy based on the Cooper regimen

Investigator(s)	Ref No.	No. of evaluable patients	Overall response	
			No. of responses	%
Lokich and Skarin	87	10	7	70
Ramirez	88	50	34	68
Leone and Rege	89	81	51	63
Stott et al	90	10	5	50
Vaughn et al	91	46	20	43.5
Davis et al	92	74	31	42
Dorsk et al	93	20	8	40
Lemkin and Dollinger	94	20	6	30
Smalley et al	95	37	11	29
Totals		348	173	50

drugs and that patient selection variables, intensity of treatment, and data-reporting techniques all contribute to the wide range of reported results.

In general, the followup to Cooper's original report has been somewhat disappointing and no one has reproduced his 90% CR rate. Two major cooperative groups with large series have noted response rates > 60% (mostly PRs) that appear greater than could be expected with single agents. Other groups have reported less impressive results and almost all would agree that Cooper's five-drug combination is still a long way from exploiting the optimum therapeutic potential of the compounds utilized.

There is little evidence of any activity by VCR in large bowel cancer. Nevertheless, it has been included in several interesting combinations. Falkson and his associates (96) recently reported a 43% response rate with a four-drug combination of VCR, 5-FU, BCNU, and DTIC. Moertel et al (97) have studied a three-drug combination of VCR, 5-FU, and methyl-CCNU and reported a 43.5% response rate showing statistically significant superiority over a concomitant control of 5-FU alone (19.5%).

VCR alone has only minimal activity in malignant melanoma with a cumulative response rate of 12% (six of 52 patients) (98). There have been some hints that VCR may be valuable in combination approaches and the drug has been extensively used in this manner.

Moon's original study (99) reporting a high response rate in melanoma with the combination of BCNU and VCR was not substantiated by a large controlled study of the ALGB (100) that compared the combination to two dose schedules of DTIC. Overall, the three studies (99–101) show a 20% response rate in 86 patients treated with BCNU and VCR. The Western Cancer Study Group (102) has found the combination of CCNU and VCR to be significantly superior to CCNU alone (20% versus 4%) but again the response rate does not appear to be superior to the extensive experience with DTIC which is the standard drug for this tumor (98).

BCNU, VCR, and DTIC (BVD) have been combined in four separate studies (table 6) (103–105).[4] The Southwest Oncology Group (SWOG) sequentially studied two different doses of the combination without obtaining an apparent difference in the response rates. Currently, the study is undergoing definitive analysis and it is not known whether there is a difference in duration of response or an increase in the responsiveness of visceral disease.

Three of the four BVD studies used comparable doses and schedules, whereas the study recently reported by Cohen et al (105) used somewhat more intensive courses of DTIC and BCNU. This preliminary report showed responses in ten of 16 patients, with seven of the responders having nodal and subcutaneous disease. This more intensive approach has not been evaluated in a controlled fashion against the less intensive three-drug combinations. Therefore, it is difficult to assess whether patient-selection factors or the higher doses constituted the critical variable in this small study.

Podophyllotoxins

Podophyllin is a resin obtained from the dried roots and rhizomes of two plant species, *Podophyllum peltatum* (the American species) or *Podophyllum emodi* (the Indian species). Both species are herbaceous perennials. *P. peltatum* is indigenous to the US and Canada, growing commonly in eastern North America, and is usually called the "may apple" or "American mandrake" (2). The American Indians knew of its properties as a cathartic, antihelmintic, and poison. The colonists used the root as a cathartic, emetic, and cholagogue. *P. emodi* resembles *P. peltatum* and grows in the interior

[4]Luce JK. Personal communication.

TABLE 6.—Activity of BVD regimens against malignant melanoma

Investigator(s)	Ref No.	Drug	Regimen	No. of evaluable patients	Overall response	
					No. of responses	%
Luce et al	footnote 4, 103	DTIC	100 or 150 mg/m²/day × 5	79	18	23
		BCNU	100 or 150 mg/m²/day × 1			
		VCR	1.4 mg/m² Days 1 and 4			
Luce	footnote 4	DTIC	30 mg/m² Day 1; then 80 or 120 mg/m²/day × 4	62	18	29
		BCNU	80 or 120 mg/m²/day × 1			
		VCR	1.5 mg/m² Days 1 and 4			
Beretta et al	104	DTIC	100 mg/m²/day × 5	41	8	20
		BCNU	100 mg/m²/day × 1			
		VCR	1.4 mg/m² Days 1 and 4			
Cohen et al	105	DTIC	400 mg/day × 3–4	16	10	63
		BCNU	100 mg/day × 3–4			
		VCR	1–1.5 mg/wk			

TABLE 7.—Compounds isolated from *Podophyllum* species (4)

Compound	Isolated from—		
	P. peltatum	*P. emodi*	*P. sikkimensis*
Podophyllotoxin	+	+	–
α-Peltatin	+	–	–
β-Peltatin	+	–	–
4′-Demethylpodophyllotoxin	+	+	–
Desoxypodophyllotoxin	+	–	–
Dehydrophodophyllotoxin	+	–	–
Sikkimotoxin	–	–	+
Podophyllotoxin glucoside	+	+	–
Picropodophyllin glucoside	+	+	–
α-Peltatin glucoside	+	–	–
β-Peltatin glucoside	+	–	–
4′-Demethylpodophyllotoxin glucoside	+	+	–

ranges of the Himalaya mountains from Sikkim to Hazara where the natives have long been familiar with the cathartic properties of the aqueous extract of the roots.

In 1942 Kaplan reported that the topical application of podophyllin in oil gave very satisfactory results in the treatment of condyloma acuminata which is a type of venereal wart (106). This was soon followed by a report of King and Sullivan (107) demonstrating that podophyllin caused pronounced cytologic changes in normal human and rabbit skin.

The many compounds that have been isolated from podophyllin fall into two chemical classes—lignans and the flavonol pigments. While the flavonols have marked biochemical and biologic reactions, their implication in antimitotic or tumor-damaging actions has been little and the most interest from the standpoint of oncology has been centered on the lignans. From 1880 to 1946, using the orthodox methods of isolation, extraction, fractional precipitation, and crystallization, only two pure compounds were isolated, ie, podophyllotoxin and quercetin. With the newer methods used since 1947, many new lignans have been separated and a partial list is given in table 7 (4).

Investigations with genuine aglycones and glucosides in animal experiments and clinical trials showed that they had antimitotic activity. In 1947 Sullivan and Wechsler (108) showed that podophyllin caused mitotic arrest during the metaphase. The glucosides were then found to have a higher therapeutic index than the aglycones (109,110).

The Sandoz Co, Basel, Switzerland, stimulated by the antimitotic activity of the compounds, undertook the synthesis of a series of derivatives of the podophyllum glucosides. From the numerous sub-

stances which they obtained, two compounds were selected for initial clinical trial. One was podophyllotoxin, 4,6-*O*-benzylidene-β-D-glucoside (SPG-827) and the other was podophyllic acid, 2-ethylhydrazide (SPI-77). Recently, Sandoz has developed two new derivatives with unique antitumor activity for clinical trial, VM-26 and VP-16-213.

Clinical Studies

The American literature contains only two studies on the early compounds, of which only one was performed in this country.

Newton et al (111) reported on the ethylhydrazide administered intra-arterially to 32 patients with localized malignant disease. Twelve patients with advanced malignant disease arising within or secondarily involving the pelvic cavity received the drug by intra-aortic injection via an indwelling catheter introduced through the femoral artery. The total doses ranged from 2 to 10 g over 1–23 days. One patient with carcinoma of the vulva and one with carcinoma of the ovary had major regressions of disease defined as a > 50% reduction in tumor mass. One patient with uterine carcinoma and one with colon carcinoma had minor regressions (< 50% reduction). Six other patients experienced relief of pain, hemorrhage, or discharge.

Six patients with advanced head and neck cancer were treated through the external carotid artery with doses totaling 5–6 g given over 6–14 days. One patient had a major tumor regression and two others had minor regressions. Five of these patients had previously received full-dosage supervoltage irradiation.

Eight patients with advanced cancer of the head and neck (seven previously untreated) were given the drug immediately before radiotherapy (3400–6000 rads). Although the patients were selected because of poor prognosis, four had CR as a result of the combined treatment and the other four had major regression. Six patients with malignant melanoma were given ten courses of drug through the external iliac artery. One patient had three courses, two had two courses, and the rest had one course. One patient had major regression after the first course and CR of the primary tumor and nodes after the second course. Two other patients had major regression and there were two minor regressions.

Toxicity included minimal mucosal reactions. The skin reaction in areas of concentration of the drug took the form of erythema and epilation. The preparation was highly irritating and produced pain in the field of injection, with evidence of vascular damage. Twenty-two of the 32 patients had pain during or immediately after drug injection. In the dose range used there was no effect on the peripheral blood counts.

Vaitkevicius and Reed (112) reported treating 16 patients iv with the ethylhydrazide derivative and 17 patients orally with the benzylidene glucoside. The ethylhydrazide was dissolved in 250 ml of normal saline and infused iv over a 2-hour period. The dose given was 15 mg/kg/day until toxicity or tumor progression forced the interruption of therapy. The dose of the benzylidene glucoside was 6–10 mg/kg/day as 25-mg capsules.

Of the 16 patients treated with the ethylhydrazide, only one with colorectal carcinoma had a measurable decrease in tumor size. Chemical thrombophlebitis occurred frequently and made daily iv injections difficult. Hematologic toxicity was observed in seven patients who received ≥ 180 mg/kg of the drug. Depressed wbc and platelet counts always returned to normal promptly after cessation of therapy; leukopenia was more common than thrombocytopenia. Hypotension occurred infrequently and only if the drug was infused rapidly. The toxic effects are outlined in table 8.

Objective responses were seen in four of the 17 patients treated with benzylidene glucoside (table 9). Severe nausea and diarrhea, or both, was seen in all of the patients and therapy had to be discontinued even in the four patients who were responding.

The authors concluded from their data that ethylhydrazide and benzylidene glucoside were distinctly different drugs. The ethylhydrazide given in large amounts produced hematologic toxicity; this did not

TABLE 8.—Toxic effects of SPI-77

Toxic effect	No. of patients	Smallest toxic dose (mg/kg)	
		Daily	Total
Thrombophlebitis	13	15	45
Hypotension	3	15	30
Leukopenia	6	15	180
Thrombocytopenia	3	20	180
Hypertension	1	30	150
Diarrhea	1	54	300

TABLE 9.—Responses obtained with SPG-827

Tumor	No. of patients	No. of objective responses
Adenocarcinoma, colon and rectum	7	1
Adenocarcinoma, ovary	3	1
Epidermoid carcinoma, bronchus	2	0
Lymphosarcoma	2	1
Cholangiocarcinoma, liver	1	1
Squamous cell carcinoma, skin	1	0
Adenocarcinoma, breast	1	0
Totals	17	4

occur in patients who had tumor regressions with the benzylidene glucoside. They felt that because of the side effects neither agent could be considered of practical therapeutic value.

Falkson, in Pretoria, South Africa, reported treating 72 far-advanced cancer patients with the ethylhydrazide or benzylidene glucoside (113). Twenty-eight received concomitant radiotherapy to a single symptomatic lesion. Twenty-two patients had received radical radiotherapy > 3 months prior to treatment; 17 patients had prior cancer chemotherapy and five had received concomitant cyclophosphamide. The ethylhydrazide was given iv (400–2000 mg/day) to a total dose as large as 17.6 g. The benzylidene glucoside was given in oral doses of 25–500 mg/day and the largest total dose was 92 g. Of the 72 patients, 36 received the benzylidene glucoside, four received the ethylhydrazide, and 32 received both agents. The largest total combined dose was 106.9 g.

Twelve of the 72 patients achieved objective responses as follows: esophagus (one of 14 patients), mesothelioma (two of six), breast (one of five), kidneys (one of two), testicular (one of four), prostate (two of four), sarcoma (two of five), stomach (one of six), and neuroblastoma (one of one).

Diarrhea proved to be the limiting factor in the administration of the benzylidene glucoside. Many patients complained of nausea but no other signs of toxicity were observed. Large doses in iv infusions of the ethylhydrazide caused some bizarre subjective sensations lasting for only a few minutes and varying from patient to patient. Large doses caused a slight fall in the wbc count.

Further studies with the early drugs have not been performed in the US but the two newer analogs have been more extensively evaluated.

Clinical studies of VM-26 have been conducted in the US and Europe. Muggia et al (114) in their phase I study noted clinical and functional improvement in two of seven patients who were evaluable on the basis of having received an adequate course of therapy. Both patients had brain tumors, which is interesting in view of the lipid solubility of VM-26 and its activity in ic inoculated L1210 mice. Dombernowsky et al (115) reported three PRs among 16 patients with Hodgkin's disease treated > 3 weeks at doses leading to toxicity. The duration of remission ranged from 60 to 90 days. Significantly, these patients had been previously treated with alkylating agents, vinca alkaloids, and procarbazine indicating that VM-26 apparently exhibits no clinical cross resistance with these agents.

Studies at the Sloan-Kettering Institute (116) produced two PRs among four patients with Hodgkin's disease and lower peripheral blood counts in one patient with ALL and in one with chronic myelocytic leukemia. Only symptomatic responses or none at all occurred in reticulum cell sarcoma and assorted tumors.

A large phase II study of VM-26 by the European Organization for Research on Treatment of Cancer (EORTC) emphasized lymphomas and other solid tumors (117). In 22 patients with Hodgkin's disease they found one CR (stage IIIA disease) of brief duration (30 days) and six PRs. VM-26 apparently was fairly active in reticulum cell sarcoma (13 PRs among 25 patients [52%]) but nine patients had no prior therapy and two had only antecedent radiotherapy. There were six PRs among 19 patients with lymphosarcomas, with striking regression of pleural effusions in three. The EORTC study of solid tumors included 119 patients but only four tumor types were represented by a sufficient number of patients to assess drug activity. The responses were one CR and six PRs, including cancer of the bladder (one CR and four PRs among 24 patients) and breast (two PRs among 22 patients); 12 patients with esophageal cancer and 16 with head and neck cancer did not respond. On the basis of this investigation, VM-26 has antitumor activity against Hodgkin's disease and non-Hodgkin's lymphomas, especially reticulum cell sarcoma. There also is interesting activity in bladder carcinoma but only minimum efficacy in breast cancer and little activity in esophagus and head and neck cancers.

In primary brain tumors VM-26 produced objective neurologic improvement in four of 12 patients having symptomatic disease and only one progression in seven asymptomatic patients (118). Responses occurred in two patients who had experienced progressive disease while undergoing therapy with nitrosoureas. Henderson[5] also investigated VM-26 without observing response in 14 patients with ALL (12 children and two adults). He did note a reduction in circulating blast cells in three patients and one PR in a child among seven acute myelocytic leukemia patients (five adults and two children).

Thus, VM-26 shows definite antitumor activity in lymphomas and brain tumors. However, except for the minimal effect in breast cancer, there is not enough information warranting further comment on the other signal solid tumors (colon, melanoma, pancreas, lungs, and ovaries). There appears to be potential use of VM-26 in primary combination therapy of lymphomas, particularly reticulum cell sarcoma. VM-26 is not unduly myelosuppressive and, otherwise, shows overlapping toxicity with the vinca alkaloids only in alopecia. At this time, none of the

[5]Personal communication.

lymphomas appear to exhibit cross resistance between VM-26 and the standard therapeutic agents used against them.

Nissen et al (119) conducted a phase I trial of VP-16-213. The drug was diluted in 250 ml of 5% glucose and infused over 30 minutes. A modified Fibonacci search scheme as described by Hansen et al (120) was used to determine the MTD. Two schedules were studied: daily iv for 7 days and twice weekly iv for 3 weeks. With the daily schedule no significant hematologic toxicity occurred until a dose of 45 mg/m^2 was given where reversible moderate leukopenia without thrombocytopenia was observed. At the next step (54 mg/m^2) severe hematologic toxicity (wbc and platelet counts) was found with a nadir 12–16 days after the initiation of treatment and recovery at Days 20–22. Consequently, a dose of 45 mg/m^2 was felt to be the MTD. Interestingly, at the lower dose levels an increase in wbc and platelet counts was seen for the first 5–9 days, after which they fell to sub-toxic levels. Nausea and vomiting was observed in a few patients at the lower dose level. No hepatic, renal, or local (phlebitis) toxicity was noted.

Using the twice weekly schedule, significant but reversible hematologic toxicity was seen at 69 and 86 mg/m^2; the latter dose was felt to be the MTD, although this study was biweekly for only 3 weeks. The authors felt that if therapy is anticipated for > 3 weeks the dose should be reduced to 54 mg/m^2 twice weekly. No other toxicity except nausea was found with this schedule.

An EORTC phase II study used VP-16-213 at 60 mg/m^2 daily for 5 days followed by a 2-week rest period;[6] two to six cycles were administered. Reports indicated that myelosuppression, mainly granulocytopenia and to a lesser extent thrombocytopenia, was the predominant toxicity. Alopecia was seen as well as anorexia and nausea and vomiting. Headache, fever, and hypotension were recorded infrequently. Of 250 patients, only nine had severe toxicity and 114 showed no signs of any toxicity.

In data accumulated from 353 patients treated in Europe[6] the only significant toxicities were reversible bone marrow suppression in approximately 85% of the patients. Rare toxicities in the range of 1%–2% were nausea and vomiting, diarrhea, hypotension, headache, fever, stomatitis, alopecia, and transient paresthesia. Doses of 90–100 mg/m^2 twice weekly or 60 mg/m^2/day × 5 every 3 weeks were considered safe and effective in patients with good bone marrow reserve. Dilutions of the iv preparation in 250 ml of saline avoided local tissue reactions.

During recent clinical trials in this country there have been reports of six cases of severe wheezing and bronchospasm which responded to cessation of infusions or use of antihistamines (121).[6]

The largest clinical experience with VP-16-213 has been in the EORTC phase I trials (119) where a significant number of responses were noted in previously treated Hodgkin's and non-Hodgkin's lymphomas and in leukemias, notably monocytic leukemia which is notoriously resistant to treatment. Few responses were seen in solid tumors, although several patients showed no disease progression while under treatment. Of great interest is the fact that some of the responses were seen without concurrent hematologic toxicity. Activity seen in patients treated with the oral preparation appeared similar to that by the parenteral route, although there was variability of absorption.

Current phase II trials are using dose schedules of 60–90 mg/m^2 twice weekly or 200–300 mg/m^2/week and include a broad phase II trial in advanced cancer by the ALGB and a phase I–II trial in advanced breast cancer by the Mayo Clinic, Rochester, Minn.

CONCLUSION

Plant products have been used to treat cancer patients for many years and could be considered historically to be the first anticancer drugs. Today, the vinca alkaloids are part of every medical oncologist's resources and play an important role in treating diverse diseases such as the leukemias, lymphomas, breast and testicular cancers, pediatric solid tumors, and malignant melanoma. They are a vital part of most of the successful combination regimens that have led to the major therapeutic triumphs in chemotherapy. The podophyllotoxins have shown evidence of activity in the leukemias and lymphomas, and possibly, in brain tumors and breast cancer. Further studies now in progress should soon delineate their role.

The active natural products program of the Drug Research and Development Program, DCT, continues to uncover new active structures and several new compounds are now in early clinical trial. These include thalicarpine and *d*-tetrandrine. Several more are nearing entry into clinical study. Perhaps in the future the plants will play an even larger role than their current highly significant one.

LIST OF COMPOUNDS

ABVP = adriamycin, bleomycin, VBL, and DTIC
Actinomycin D: NSC-3053

[6]Handelsman H, Goldsmith MA, and Slavik M. Clinical brochure: 4′-demethylepipodophyllotoxin-β-D-ethylidene glucoside (NSC-141540) VP 16-213. Prepared by the Cancer Therapy Evaluation Program, DCT, NCI, Bethesda, Md, Apr 1974.

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BCVPP = BCNU, cyclophosphamide, VBL, procarbazine, and prednisone

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

BVD = BCNU, VCR, and DTIC

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cooper regimen = 5-FU, methotrexate, VCR, cyclophosphamide, and prednisone

CVP = cyclophosphamide, VCR, and prednisone

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; Mustargen; nitrogen mustard

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

MOPP = mechlorethamine, VCR (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11-20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

SPG-827: NSC-42076

SPI-77: NSC-72274

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate; Velban; VBL

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin; VCR

VP-16-213: NSC-141540; 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside); PTG

VM-26: NSC-122819; 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-thenylidene-β-D-glucopyranoside)

REFERENCES

1. BELKIN M. Effect of podophyllin on transplanted mouse tumors. Fed Proc 6:308, 1947.
2. HARTWELL JL, and SHEAR MJ. Chemotherapy of cancer: classes of compounds under investigation and active components of podophyllin. Cancer Res 7:716, 1947.
3. LOMBARDI LH. Medicinal plants of our Maine Indians. *In* The Maine Writers Research Club. Maine Indians in History and Legends. Portland, Me, Severn-Wylie-Jewett, 1952.
4. HARTWELL JL, and SCHRECKER AW. The chemistry of podophyllum. Vienna, Springer-Verlag, 1958.
5. SAVEL H. The metaphase-arresting plant alkaloids and cancer chemotherapy. Prog Exp Tumor Res 8:189–224, 1966.
6. NOBLE RL, BEER CT, and CUTTS JH. Role of chance observations in chemotherapy: *Vinca rosea*. Ann NY Acad Sci 76:882–894, 1958.
7. BEER CT. The leucopenic action of extracts of *Vinca rosea*. *In* 33rd Annual Report of the British Empire Cancer Campaign, 1955, pp 487–488.
8. CUTTS JH, BEER CT, and NOBLE RL. Effects on hematopoiesis in rats of extracts of *Vinca rosea*. Rev Can Biol 16:476, 1957.
9. JOHNSON IS, WRIGHT HF, and SVOBODA GH. Experimental basis for clinical evaluation of antitumor principles derived from *Vinca rosea* Linn. J Lab Clin Med 54:830, 1959.
10. ———. Antitumor principles derived from *Vinca rosea* Linn. Proc Am Assoc Cancer Res 3:122, 1960.
11. JOHNSON IS, ARMSTRONG JG, GORMAN M, ET AL. The vinca alkaloids: a new class of oncolytic agents. Cancer Res 23:1390–1427, 1963.
12. CREASEY WA. Modifications in biochemical pathways produced by the vinca alkaloids. Cancer Chemother Rep 52:501–507, 1968.
13. MADOC-JONES H, and MAURO F. Interphase action of vinblastine and vincristine: differences in their lethal action through the mitotic cycle of cultured mammalian cells. J Cell Physiol 72:185–196, 1968.
14. BENSCH KG, and MALAWISTA SE. Microtubular crystals in mammalian cells. J Cell Biol 40:95–107, 1969.
15. CARTER SK, and LIVINGSTON RB. Single-agent therapy for Hodgkin's disease. Ann Intern Med 131:377–387, 1973.
16. CARBONE PP, SPURR C, and SCHNEIDERMAN M. Management of patients with malignant lymphoma: a comparative study with cyclophosphamide and vinca alkaloids. Cancer Res 28:811–822, 1968.
17. STUTZMAN L, EZDINLI EZ, and STUTZMAN MA. Vinblastine sulfate vs cyclophosphamide in the therapy for lymphoma. JAMA 195:173–178, 1966.
18. EZDINLI EZ, and STUTZMAN L. Vinblastine vs nitrogen mustard therapy of Hodgkin's disease. Cancer 22:473–479, 1968.
19. WARWICK OH, ALISON RE, and DARTE JMM. Clinical experience with vinblastine sulfate. Can Med Assoc J 85:579–583, 1961.
20. WHITELAW DM, and TEASDALE JM. Vincaleukoblastine in the treatment of malignant disease. Can Med Assoc J 85:584–591, 1961.
21. HODES M, ROHN RJ, BOND WH, ET AL. Vincaleukoblastine. IV. A summary of two and one-half years' experience in the use of vinblastine. Cancer Chemother Rep 16:401–406, 1962.
22. ARMSTRONG JG, DYKE RW, FOUTS PJ, ET AL. Hodgkin's disease, carcinoma of the breast, and other tumors treated with vinblastine sulfate. Cancer Chemother Rep 18:49–71, 1962.
23. FROST JW, GOLDWEIN MI, and BRYAN JA. Clinical experience with vincaleukoblastine in far-advanced Hodgkin's disease and various malignant states. Ann Intern Med 56:854–859, 1962.
24. MIDWEST COOPERATIVE CHEMOTHERAPY GROUP. Vinblastine in neoplastic disease. Cancer Res 23:169–179, 1963.
25. MARSDEN JA. Report on the use of vincaleukoblastine in 14 cases of Hodgkin's disease. Med J Aust 2:100–102, 1963.
26. BERNARD J, JACQUILLAT C, and BOIRON M. Traitement de la maladie de Hodgkin par la vincaleukoblastine: a propos de 85 observations. Presse Med 72:1471–1476, 1964.
27. BUTLER KR. Clinical experience with vincaleukoblastine sulfate in the treatment of 11 patients with Hodgkin's disease. Can Med Assoc J 93:735–739, 1965.
28. EASTERN COOPERATIVE GROUP IN SOLID TUMOR CHEMOTHERAPY AND ACUTE LEUKEMIA COOPERATIVE GROUP B. Neoplastic disease. Treatment with vinblastine. Arch Intern Med 116:846–852, 1965.
29. BOND WH, ROHN RJ, BATES LH, ET AL. Treatment of neoplastic disease with an improved oral preparation of vinblastine sulfate. Cancer 19:213–219, 1966.
30. MACDONALD CA, JR, and LACHER MJ. Oral vinblastine sulfate in Hodgkin's disease. Clin Pharmacol Ther 7:534–541, 1966.
31. VIALA JJ, REVOL L, and CROIZAT P. Two years' experience in

Lyons with vincaleukoblastine, especially in Hodgkin's disease. *In* Antitumor Effects of *Vinca rosea* Alkaloids (Garattini S, and Sproston EM, eds). New York, Excerpta Medica Foundation, 1966, pp 114–118.
32. SOHIER WD, JR, WONG RKL, and AISENBERG AC. Vinblastine in the treatment of advanced Hodgkin's disease. Cancer 22:467–472, 1968.
33. ANGLESIO E, CALCIATI A, and CARAMELLO V. The treatment of lymphomas with vincaleukoblastine and methylhydrazine. Acta Genet Med (Roma) 17:232–244, 1968.
34. FLATOW FA, ULTMANN JE, and HYMAN GA. Treatment of advanced Hodgkin's disease with vinblastine (NSC-49842) or procarbazine (NSC-77213). Cancer Chemother Rep 53:39–47, 1969.
35. JELLIFFE AM. Vinblastine in the treatment of Hodgkin's disease. Br J Cancer 23:44–48, 1969.
36. LIVINGSTON RB, and CARTER SK. Single Agents in Cancer Chemotherapy. New York, IFI/Plenum, 1970.
37. HERTZ R, LIPSETT MB, and MOY RH. Effect of vincaleukoblastine on metastatic choriocarcinoma and related trophoblastic tumors in women. Cancer Res 20:1050–1058, 1960.
38. CARTER SK. Single and combination nonhormonal chemotherapy in breast cancer. Cancer 30:1543–1555, 1972.
39. JOHNSTON B, and NOVALES ET. The use of vinblastine sulfate (Velban) in advanced malignancies of the female reproductive tract. Proc Am Assoc Cancer Res 5:32, 1964.
40. BLEEHEN NM, and JELLIFFE AM. Vinblastine sulfate in the treatment of malignant disease. Br J Cancer 19:268–273, 1965.
41. GOLDENBERG I. Vinblastine sulfate (VLB) therapy of women with advanced breast cancer. Cancer Chemother Rep 29:111–113, 1963.
42. SAMUELS ML, and HOWE CD. Vinblastine in the management of testicular cancer. Cancer 25:1009–1017, 1970.
43. SMART CR, ROCHLIN DB, NAHUM AM, ET AL. Clinical experience with vinblastine sulfate (NSC–49842) in squamous cell carcinoma and other malignancies. Cancer Chemother Rep 34:31–45, 1964.
44. SAMUELS ML, JOHNSON DE, and HOLOYE PY. The treatment of stage 3 metastatic germinal cell neoplasia of the testis with bleomycin combination chemotherapy. Proc Am Assoc Cancer Res 14:23, 1973.
45. SAMUELS ML. Continuous intravenous bleomycin therapy with vinblastine in testicular and extragonadal germinal tumors. Proc Am Assoc Cancer Res and ASCO 16:112, 1975.
46. SILVAY O, YAGODA A, WITTES R, ET AL. Treatment of germ cell carcinomas with a combination of actinomycin D, vinblastine, and bleomycin. Proc Am Assoc Cancer Res 14:68, 1973.
47. JOURNEY LJ, BURDMAN J, and WHALEY A. Electron microscopic study of spinal ganglia from vincristine-treated mice. J Natl Cancer Inst 43:603–619, 1969.
48. UY QL, JOHNS RJ, and OWENS AH, JR. Vincristine (VCR) neurotoxicity in mice. Fed Proc 25:454, 1966.
49. TOBIN W, and SANDLER SG. Vincristine-induced neuropathy: localization of the early neurophysiologic lesion. Proc Am Assoc Cancer Res 7:71, 1966.
50. GOTTSCHALK PG, DYCK PJ, and KIELY JM. Vinca alkaloid neuropathy: nerve biopsy studies in rats and man. Neurology 18:875–882, 1968.
51. WEISS HD, WALKER MD, and WIERNIK PH. Neurotoxicity of commonly used antineoplastic agents. N Engl J Med 291:127–133, 1974.
52. SANDLER SG, TOBIN W, and HENDERSON ES. Vincristine-induced neuropathy: a clinical study of fifty leukemic patients. Neurology 19:367–374, 1969.
53. BRADLEY WG, LASSMAN LP, PEARCE GW, ET AL. The neuromyopathy of vincristine in man: clinical, electrophysiological and pathological studies. J Neurol Sci 10:107–131, 1970.
54. CASEY EB, JELLIFFE AM, LEQUESNE PM, ET AL. Vincristine neuropathy. Clinical and electrophysiological observations. Brain 96:69–86, 1973.
55. HOLLAND JF, SCHARLAU C, GAILANI S, ET AL. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.
56. GOTTLIEB RJ, and CUTTNER J. Vincristine-induced bladder atony. Cancer 28:674–675, 1971.
57. CARMICHAEL SM, EAGLETON L, AYERS CR, ET AL. Orthostatic hypotension during vincristine therapy. Arch Intern Med 126:290–293, 1970.
58. HENDERSON ES. Acute lymphoblastic leukemia. *In* Cancer Medicine (Frei EJ, and Holland J, eds). Philadelphia, Lea & Febiger, 1973, pp 1173–1199.
59. BRODER L, and CARTER SK. An overview of therapy of acute leukemia. *In* Meningeal Leukemia (Broder L, and Carter SK, eds). New York, Plenum Press, 1972.
60. SULLIVAN MP. Vincristine (NSC-67574) therapy for Wilms' tumor. Cancer Chemother Rep 52:481–484, 1968.
61. GREENWALD ES. Cancer chemotherapy. II. NY State J Med 38:2641–2654, 1972.
62. JAMES DH, and GEORGE P. Vincristine in children with malignant solid tumors. J Pediatr 64:534–541, 1964.
63. SUTOW WW. Vincristine (NSC-67574) therapy for malignant solid tumors in children (except Wilms' tumor). Cancer Chemother Rep 52:485–487, 1968.
64. EDLAND RW. Embryonal rhabdomyosarcoma. Am J Roentgenol 93:671–685, 1965.
65. CARBONE PP, BONO V, FREI E III, ET AL. Clinical studies with vincristine. Blood 21:640–647, 1963.
66. WHITELAW DM, and KIM HS. Vincristine in the treatment of neoplastic disease. Can Med Assoc J 90:1385–1389, 1964.
67. BOHANNON RA, MILLER DG, and DIAMOND HD. Vincristine in the treatment of lymphomas and leukemias. Cancer Res 23:613–621, 1963.
68. MARTIN J, and COMPSTON N. Vincristine sulphate in the treatment of lymphoma and leukemia. Lancet 2:1080–1083, 1963.
69. GAILANI S. Phase II studies of vincristine (VCR) in human cancer. Proc Am Assoc Cancer Res 4:21, 1963.
70. SELAWRY OS, HOLLAND JF, and WOLMAN IJ. Effect of vincristine (NSC-67574) on malignant solid tumors in children. Cancer Chemother Rep 52:497–500, 1968.
71. DESAI D, EZDINLI EZ, and STUTZMAN L. Vincristine (VCR) therapy of lymphoma and chronic lymphocytic leukemia (CLL). Proc Am Assoc Cancer Res 10:18, 1969.
72. LACHER MJ, and DURANT JR. Combined vinblastine and chlorambucil therapy of Hodgkin's disease. Ann Intern Med 62:468–476, 1965.
73. MOXLEY JH, JR, DEVITA VT, BRACE K, ET AL. Intensive combination chemotherapy and X-irradiation in Hodgkin's disease. Cancer Res 27:1258–1263, 1967.
74. DEVITA VT, and SERPICK A. Combination chemotherapy in the treatment of advanced Hodgkin's disease (HD). Proc Am Assoc Cancer Res 8:13, 1967.
75. DEVITA VT, SERPICK A, and CARBONE PP. Combination chemotherapy of advanced Hodgkin's disease (HD): the NCI program, a progress report. Proc Am Assoc Cancer Res 10:19, 1969.
76. ———. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881–895, 1970.
77. DEVITA VT, CANELLOS GP, SCHEIN PS, ET AL. Malignant lymphoma: treatment with combination chemotherapy. *In* Proceedings of the 7th National Cancer Conference, Los Angeles, Calif, Sept 27–29, 1972. Philadelphia, J. B. Lippincott Co, 1972, pp 379–390.
78. FREI E III, DEVITA VT, MOXLEY JH, ET AL. Approaches to improving the chemotherapy of Hodgkin's disease. Cancer Res 26:1284–1289, 1966.

79. GOLDSMITH MA, and CARTER SK. Combination chemotherapy of advanced Hodgkin's disease—a review. Cancer 33:1–8, 1974.
80. BAGLEY CM, DEVITA VT, JR, BERARD CW, ET AL. Advanced lymphosarcoma: intensive cyclical combination chemotherapy with cyclophosphamide, vincristine, and prednisone. Ann Intern Med 76:227–234, 1972.
81. SCHEIN PS, CHABNER BA, CANELLOS GP, ET AL. Potential for prolonged disease-free survival following combination chemotherapy of non-Hodgkin's lymphoma. Blood 43:181–189, 1974.
82. DEVITA VT, JR, CANELLOS GP, and CHABNER B. Advanced diffuse histiocytic lymphoma, a potentially curable disease. Lancet 1(7901):248–250, 1975.
83. GRINBERG R, NEMOTO T, and DAO TL. Vincristine (NSC-67574): dosage and response in advanced breast cancer. Cancer Chemother Rep 45:57–61, 1965.
85. GOLDENBERG IS. Vincristine (NSC-67574) therapy of women with advanced breast cancer. Cancer Chemother Rep 41:7–9, 1964.
85. SILVA ARM, SMART CR, and ROCHLIN DB. Chemotherapy of breast cancer. Surg Gynecol Obstet 121:494–498, 1965.
86. COOPER R. Combination chemotherapy in hormone resistant breast cancer. Proc Am Assoc Cancer Res 10:15, 1969.
87. LOKICH JJ, and SKARIN AT. Five-drug combination chemotherapy for disseminated adenocarcinoma. Cancer Chemother Rep 56:761–767, 1972.
88. RAMIREZ G. Five-drug combination therapy in the treatment of solid tumors. Proc Am Assoc Cancer Res 14:17, 1973.
89. LEONE LA, and REGE V. Treatment of metastatic, recurrent or inoperable carcinoma of breast with VCR/PRED/5-FU/MTX/CYCLO (reg I) vs. VCR/PRED/5-FU (reg II). Proc Am Assoc Cancer Res 14:125, 1973.
90. STOTT PB, ZELKOWITZ L, and TUCKER WG. Combination chemo-hormonal therapy for disseminated breast carcinoma. (Abstr 66) Cancer Chemother Rep 57:106, 1973.
91. VAUGHN CB, BAKER LH, AL-SARRAF M, ET AL. Combination vs sequential cytotoxic chemotherapy in the treatment of advanced breast cancer. (Abstr 87) Cancer Chemother Rep 57:111, 1973.
92. DAVIS HL, JR, RAMIREZ G, ELLERBY RA, ET AL. Five-drug therapy in advanced breast cancer. Factors influencing toxicity and response. Cancer 34:239–245, 1973.
93. DORSK BM, KAUFMAN RJ, WINN RJ, ET AL. Combination chemotherapy with five drugs in advanced metastatic breast cancer. Proc Am Assoc Cancer Res 13:30, 1972.
94. LEMKIN SR, and DOLLINGER MR. Combination vs single drug therapy in advanced breast cancer. Proc Am Assoc Cancer Res 14:37, 1973.
95. SMALLEY RV, MURPHY S, CHAN Y-K, ET AL. Comparison of two five drug regimens vs. sequential chemotherapy in metastatic breast carcinoma. (Abstr 82) Cancer Chemother Rep 57:110, 1973.
96. FALKSON G, VAN EDEN EB, and FALKSON HC. Fluorouracil, imidazole carboxamide dimethyl triazeno, vincristine, and bis-chloroethyl nitrosourea in colon cancer. Cancer 33:1207–1209, 1974.
97. MOERTEL CG, SCHUTT AJ, HAHN RG, ET AL. Therapy of advanced colorectal cancer with a combination of 5-fluorouracil, methyl-1,3-*cis*(2-chloroethyl)-1-nitrosourea, and vincristine. J Natl Cancer Inst 54:69–71, 1975.
98. COMIS RL, and CARTER SK. Integration of chemotherapy into combined modality therapy of solid tumors. IV. Malignant melanoma. Cancer Treat Rev 1:285–304, 1974.
99. MOON JH. Combination chemotherapy in malignant melanoma. Cancer 26:468–473, 1970.
100. GAILANI S, and MOON J. Comparative study of imidazole carboxamide dimethyl triazeno (ICDT) and combination of BCNU and vincristine in treatment of malignant melanoma. (Abstr 23) Proc Am Soc Clin Oncol, 1971.
101. MARSH JC, DECONTI RC, and HUBBARD SP. Treatment of Hodgkin's disease and other cancers with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962). Cancer Chemother Rep 55:599–606, 1971.
102. PUGH R, JACOBS E, BATEMAN J, ET AL. *In* Proceedings of the 11th International Cancer Congress, Florence, Italy, Oct 20–26, 1974.
103. LUCE JK, TORIN LB, and PRICE H. Combination dimethyl triazeno imidazole carboxamide (NSC 45388; DIC), vincristine (NSC 67574; VCR) and 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962; BCNU) chemotherapy of disseminated malignant melanoma. Proc Am Assoc Cancer Res 11:50, 1970.
104. BERETTA G, BAJETTA E, BONADONNA G, ET AL. Polichemioterapia con 5-(3,3-dimetil-1-triazeno)-imidazole-4-carboxamide (DTIC; NSC-45388), 1,3-bis (2-cloretil)-1-nitrosourea (BCNU; NSC-409962) e vincristina (NSC-67574) nel melanoma in fase metastatizzata. Tumori 59:239–248, 1973.
105. COHEN SM, GREENSPAN EM, WEINER MJ, ET AL. Triple combination chemotherapy of disseminated melanoma. Cancer 29:1489–1495, 1972.
106. KAPLAN IW. Condylomata acuminata. N Orleans Med Surg J 94:388, 1942.
107. KING LS, and SULLIVAN M. The similarity of the effect of podophyllin and colchicine and their use in the treatment of condylomata acuminata. Science 104:244–245, 1946.
108. SULLIVAN BJ, and WECHSLER HI. The cytological effects of podophyllin. Science 105:433, 1947.
109. CORLETTI A, EMMENEGGER H, and STÄHELIN H. Recherches sur les proprietes antimitotiques de substances derivees de la podophyllotixine. Actual Pharmacol 12:103–128, 1959.
110. EMMENEGGER H, STAEHELIN H, RUTSCHMANN J, ET AL. Zur chemie und pharmakologic der podophyllum-glucoside und ihrer derivate. [The chemistry and pharmacology of the *Podophyllum* glucosides and their derivatives.] Arzneim Forsch 11:327–333, 1961.
111. NEWTON KA, WESTBURY G, and WHITE WF. Intra-arterial administration of 2-ethylhydrazide of podophyllic acid (NSC-72274) in localized malignant disease. Cancer Chemother Rep 43:33–38, 1964.
112. VAITKEVICIUS VK, and REED ML. Clinical studies with podophyllum compounds SPI-77 (NSC-72274) and SPG-827 (NSC-42076). Cancer Chemother Rep 50:565–571, 1966.
113. FALKSON G, SANDISON AG, and VAN ZYL J. Podophyllin derivatives SPG 827 and SPI 77 in the treatment of advanced cancer. S Afr J Radiol 2:1–7, 1964.
114. MUGGIA F, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
115. DOMBERNOWSKY P, NISSEN I, and LARSEN V. Clinical investigation of a new podophyllum derivative epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819) in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
116. TREMPE G, SYKES M, YOUNG C, ET AL. Phase I trial of the podophyllotoxin derivative VM-26. Proc Am Assoc Cancer Res 11:79, 1970.
117. EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER (COOPERATIVE GROUP FOR LEUKAEMIAS AND HAEMATOSARCOMAS). Clinical screening of epipodophyllotoxin VM-26 in malignant lymphomas and solid tumors. Br Med J 2:744–746, 1972.
118. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, ET AL. 4′Demethyl-epipodophyllotoxin-β-D thenylidene glucoside (PTG) in the treatment of intracranial neoplasms (phase II). (Abstr 11) Cancer Chemother Rep 57:91, 1973.

119. NISSEN NI, LARSEN V, PEDERSEN H, ET AL. Phase I clinical trial of a new agent, 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-213). Cancer Chemother Rep 56:769–777, 1972.

120. HANSEN HH, SELAWRY OS, MUGGIA FM, ET AL. Clinical studies with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037). Cancer Res 31:223–227, 1971.

121. YOUNG CW, IHDE DC, and VON STUBBE W. Preliminary clinical trial of 4′-demethyl-epipodophyllotoxin-β-D-ethylidene-glucoside (VP 16–213). Proc Am Assoc Cancer Res 14:60, 1973.

The Genus *Cephalotaxus:* Source of Homoharringtonine and Related Anticancer Alkaloids

Cecil R. Smith, Jr,* Richard G. Powell, and Kenneth L. Mikolajczak[1]

SUMMARY

Various phases of research on *Cephalotaxus* alkaloids are summarized, including their discovery, nature of antitumor activity, isolation, structural characterization, synthesis, biogenesis, and mechanism of physiologic action.

[Cancer Treat Rep 60:1157–1170, 1976]

Cephalotaxus is a genus of yew-like coniferous trees and shrubs consisting of seven species native to eastern Asia (1). This genus is the source of a unique group of alkaloids, the prototype of which is cephalotaxine (fig 1, I). The antitumor activity of several esters of cephalotaxine has generated considerable research activity along several lines, including structural determination and synthesis of these alkaloids and elucidation of their biogenesis and mechanism of physiologic action. The sequential development of these various investigations with *Cephalotaxus* serves as a "case history" in the evolution of an active plant constituent from its detection in a screening program to its recognition as a potential chemotherapeutic agent.

The antitumor activity in *Cephalotaxus* was first detected in an aqueous extract of *C. harringtonia* (Forbes) K. Koch seeds. Later, ethanol extraction was shown to be the preferred method of isolating the active principles of *Cephalotaxus*, which were found in various parts of the plant; these extracts were confirmed in both P388 and L1210 experimental leukemia systems. By a combination of countercurrent distribution and thin-layer chromatography, cephalotaxine (I) was isolated as well as four of its esters: harringtonine (fig 1, II), isoharringtonine (fig 1, III), homoharringtonine (fig 1, IV), and deoxyharringtonine (fig 1, V) (2,3). Cephalotaxine per se is inactive while these four esters exhibit various degrees of activity (3) (tables 1 and 2). Harringtonine and homoharringtonine show about the same activity in the P388 system and are both distinctly more active than deoxyharringtonine. Several times as much isoharringtonine is required to reach its maximum T/C value in comparison with values for harringtonine and homoharringtonine. These latter two alkaloids are marginally active in the L1210 system.

Characterization of Cephalotaxine

Cephalotaxine was isolated from two *Cephalotaxus* species by Paudler et al (4) who studied its chemistry extensively. Through a combination of chemical and spectral evidence, they ascertained that cephalotaxine had the empirical formula $C_{18}H_{21}NO_4$ and contained a methylenedioxyphenyl group, a secondary hydroxyl group, a double bond, and a vinylic methoxyl group. McKay (5) tentatively proposed a structure (fig 2, VII) for cephalotaxine. After it became apparent that the active compounds in *Cephalotaxus* are esters of cephalotaxine, Powell and co-workers (6) reconsidered the constitution of this alkaloid with structure VII as their point of departure. Although most of the spectral characteristics of cephalotaxine were consistent with structure VII, they noted that this structure called for a nuclear magnetic resonance (NMR) doublet signal for the olefinic proton, but the spectrum instead showed a singlet at δ 4.85. They formulated two alternative revisions, I and VIII (fig 2), which satisfied the structural implications of the NMR spectrum including placement of the olefinic proton adjacent to a quaternary carbon. Formula I was shown to correctly depict the structure and relative stereochemistry through the X-ray crystallographic work of Abraham et al (7); their crystallographic study was based on cephalotaxine methiodide.

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TABLE 1.—Activity of some *Cephalotaxus* alkaloids against P388 lymphocytic leukemia*

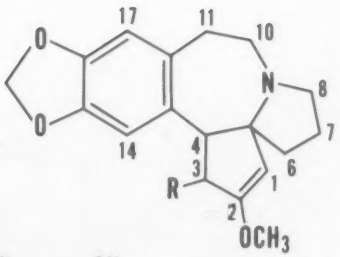
Alkaloid	Dose (mg/kg)	Survivors	Animal weight difference (T–C)	Survival time, T/C (days)	T/C (%)
II	4.00	2/6	−5.5	5.0/9.0	—
	2.00	6/6	−3.3	18.5/9.0	205
	1.00	6/6	−2.3	36.5/9.0	405
	0.50	6/6	−1.0	26.5/9.0	294
III	15.0	6/6	−4.3	9.5/9.0	105
	7.50	6/6	−3.0	24.5/9.0	272
	3.75	6/6	−2.8	15.5/9.0	172
	1.87	5/6	−1.3	13.5/9.0	150
IV	2.00	6/6	−3.8	7.5/9.0	—
	1.00	6/6	−2.8	30.5/9.0	338
	0.50	6/6	−1.8	24.5/9.0	272
	0.25	6/6	−2.2	22.0/9.0	244
V	4.00	6/6	−3.4	14.0/10.0	140
	2.00	6/6	−3.3	18.0/10.0	180
	1.00	6/6	−2.4	15.5/10.0	155
	0.50	6/6	−1.2	14.5/10.0	145

*Data presented are representative of results from several assays with different samples of each alkaloid. Materials are considered active if the survival time of animals treated (T) with them is ≥ 125% of that of the controls (C) (ie, T/C ≥ 125%). Values are quoted from ref 3.

TABLE 2.—Activity of some *Cephalotaxus* alkaloids against lymphoid leukemia L1210*

Alkaloid	Dose (mg/kg)	Survivors	Animal weight difference (T–C)	Survival time, T/C (days)	T/C (%)
I	220	6/6	−1.2	9.8/9.6	102
	110	6/6	0.2	10.3/9.6	107
	55	6/6	0.5	9.5/9.6	98
II	4.00	1/6	−4.1	0.0/9.1	—
	2.00	6/6	−2.4	12.5/9.1	137
	1.00	6/6	−1.2	12.3/9.1	135
	0.50	6/6	−1.0	12.0/9.1	131
III	15.00	6/6	−3.5	10.0/9.1	109
	7.50	6/6	−1.3	11.5/9.1	126
	3.75	6/6	−0.5	11.3/9.1	124
	1.87	6/6	−1.1	10.0/9.1	109
IV	2.00	6/6	−3.0	9.2/9.1	101
	1.00	6/6	−1.4	13.0/9.1	142
	0.50	6/6	−0.8	11.0/9.1	120
	0.25	6/6	−0.5	11.2/9.1	123
I acetate	100	6/6	−0.6	9.8/9.6	102
	50	6/6	0.0	10.2/9.6	106
	25	6/6	−0.3	10.2/9.6	106

*See footnote, table 1.



I. R = —OH

II. R = $CH_3C(CH_2)_2CCH_2CO_2CH_3$ (OH, CH_3, OH, CO_2^-)

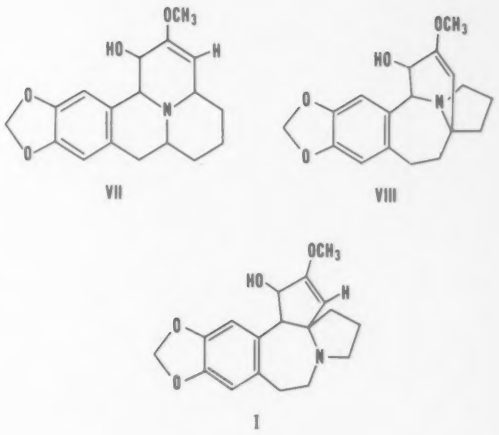
III. R = $CH_3CH(CH_2)_2C—CHCO_2CH_3$ (OH, CH_3, CO_2^-, OH)

IV. R = $CH_3C(CH_2)_3CCH_2CO_2CH_3$ (OH, CH_3, OH, CO_2^-)

V. R = $CH_3CH(CH_2)_2C—CH_2CO_2CH_3$ (OH, CH_3, CO_2^-)

VI. R = $CH_3CH(CH_2)_2CCH_2CO_2^-$ (OH, CH_3, CO_2CH_3)

FIGURE 1



VII VIII I

FIGURE 2

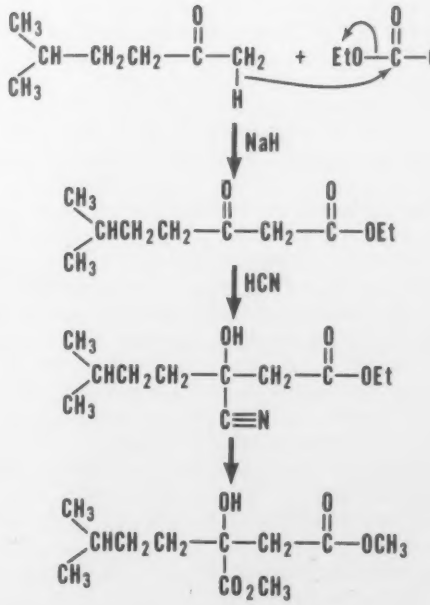


FIGURE 3

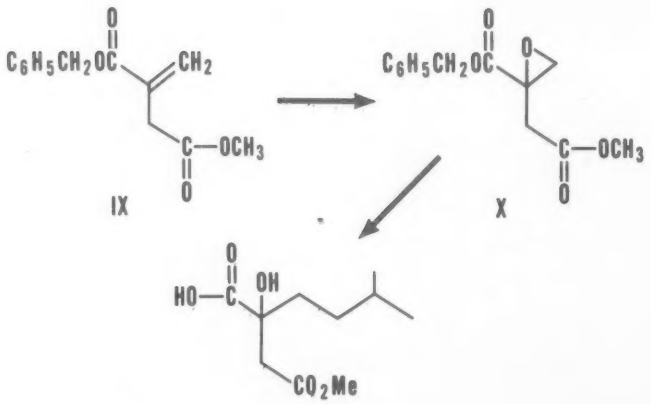


FIGURE 4

Characterization of Active Esters and Synthesis of Acid Moieties

Alkaline hydrolysis of each physiologically active ester from *Cephalotaxus* yields cephalotaxine plus a dicarboxylic acid. Initially, the dicarboxylic acid moieties from harringtonine, isoharringtonine, and homoharringtonine were identified by NMR and mass spectral studies on the derived dimethyl esters (8). Subsequently, the acid moiety of deoxyharringtonine (V) was similarly characterized and the assigned structure was verified by synthesis in the manner shown in figure 3. Characterization of these acids did not indicate which of their two carboxyl groups was attached to the cephalotaxine moiety, but an attempted synthesis of deoxyharringtonine from cephalotaxine (I) revealed that deoxyharringtonine must have the acid side chain attached through its tertiary carboxyl group (9). When cephalotaxine was acylated by the requisite half ester having the *primary* carboxyl group in the acid chloride form, the resulting product was ψ-deoxyharringtonine (fig 1, VI). The NMR spectrum of compound VI was distinctly different from that of deoxyharringtonine, thus providing evidence for structure V. From the close parallels of their NMR spectra, it was evident that the ester grouping was attached similarly in harringtonine, isoharringtonine, homoharringtonine, and deoxyharringtonine.

The structures of the dicarboxylic acid moieties of harringtonine, isoharringtonine, and deoxyharringtonine were reinforced by further confirmatory syntheses. Auerbach et al (10) carried out an alternative synthesis of the acyl group derived from deoxyharringtonine by the conjugate addition of an organo-copper reagent to the epoxide (fig 4, X) of benzyl methyl itaconate (fig 4, IX). This same research group also synthesized two diastereomeric compounds corresponding to the acid moiety of isoharringtonine (fig 5) and thereby determined that the relative configuration of its diol grouping is erythro (11). Kelly and co-workers (12) synthesized the diacid from harringtonine by a sequence which was adapted later to partial synthesis of deoxyharringtonine (V).

Absolute Configuration of Cephalotaxine and its Esters

Although the synthetic and X-ray crystallographic work we have discussed established the relative configurations of cephalotaxine and its natural esters, questions of absolute configuration were unanswered. By an X-ray crystallographic study of its *p*-bromobenzoate, Arora et al (13) demonstrated that natural (−)-cephalotaxine has the *3S,4S,5R* configuration.[2] Brandänge and co-workers (14) performed circular dichroism studies on the molybdate complex of the acid from isoharringtonine (III) (fig 6) and found that it has the *2R,3S* configuration. Similarly, the acids from harringtonine (II) and homoharringtonine (IV) were shown to have the *2R* configuration by comparing the circular dichroism spectra of their molybdate complexes with those of some synthetic *2S*-alkylmalic acids (15).

[2]Arora et al (13) followed the numbering system used for cephalotaxine in ref 6 rather than the revised scheme indicated in structure I of this paper. The numbering of chiral centers in ref 13 has been revised accordingly.

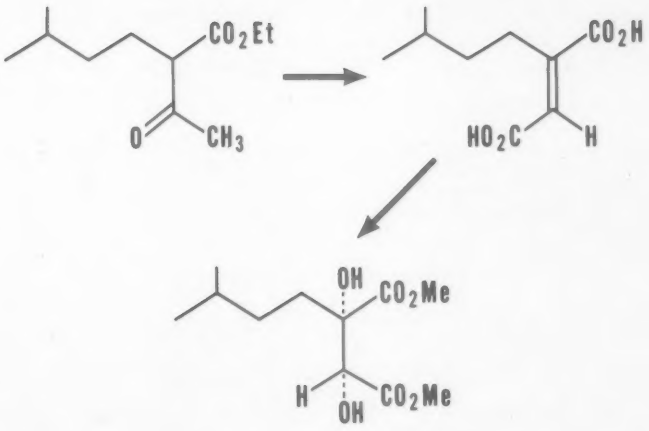


FIGURE 5

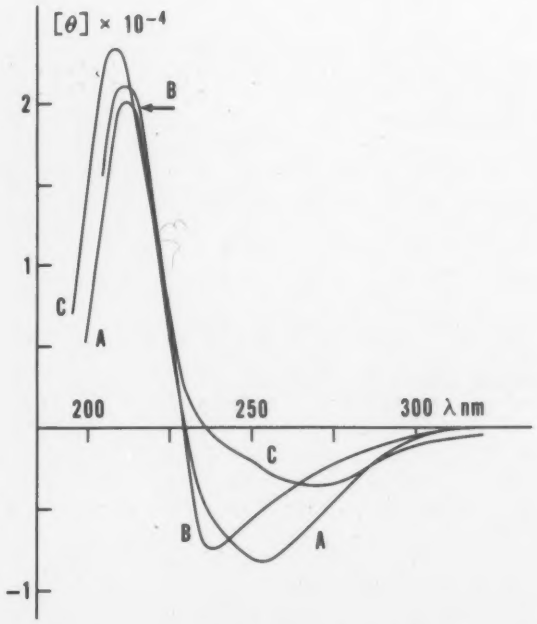


FIGURE 6.—Circular dichroism spectra of molybdate complexes of the acid from isoharringtonine (A), piscidic acid (B), and the hexahydro derivative of piscidic acid (C). The solutions had pH 2.9, 2.9, and 3.1, respectively. (From ref 14; reproduced with the consent of Acta Chemica Scandinavica.)

Syntheses of Cephalotaxine and its Active Esters

The novel character of cephalotaxine's ring system and the meager natural occurrence of its active esters have prompted considerable synthetic activity. The relative abundance of cephalotaxine, together with its lack of antitumor activity, made it particularly desirable to convert cephalotaxine into one or more of its active esters. Because it has the simplest acyl moiety, deoxyharringtonine was selected as the initial target in this effort. Preparation of this alkaloid with the acylating reagent fully elaborated might appear straightforward; however, attempts to carry out this reaction have failed consistently, apparently because of formidable steric hindrance (9,10,16). A variety of indirect approaches to the partial synthesis of deoxyharringtonine also have been tried, culminating in the successful sequence seen in figure 7. The crucial feature of this synthesis was the condensation of lithio methyl acetate ($LiCH_2CO_2CH_3$) with an α-ketoacyl cephalotaxine to provide the two-carbon branch of the ester side chain. The product contained two diastereomers which were separable by chromatography and distinguishable by NMR (16).

Weinreb and Auerbach (17) carried out a total synthesis of (±)-cephalotaxine which began by condensation of piperonal-derived compound XI with prolinol (XII) to give compound XIII, which was cyclized to give compound XIV (fig 8). Tricyclic intermediate XIV, which figured in another projected route to cephalotaxine (18), was designed for use in an enamine condensation procedure to give XV (fig 8) and thus begin construction of the fourth ring. Ultimately, XV was cyclized to give desmethylcephalotaxinone (fig 8, XVI), a naturally occurring

[3]Compound XVI will be discussed later in a section on companion alkaloids of cephalotaxine.

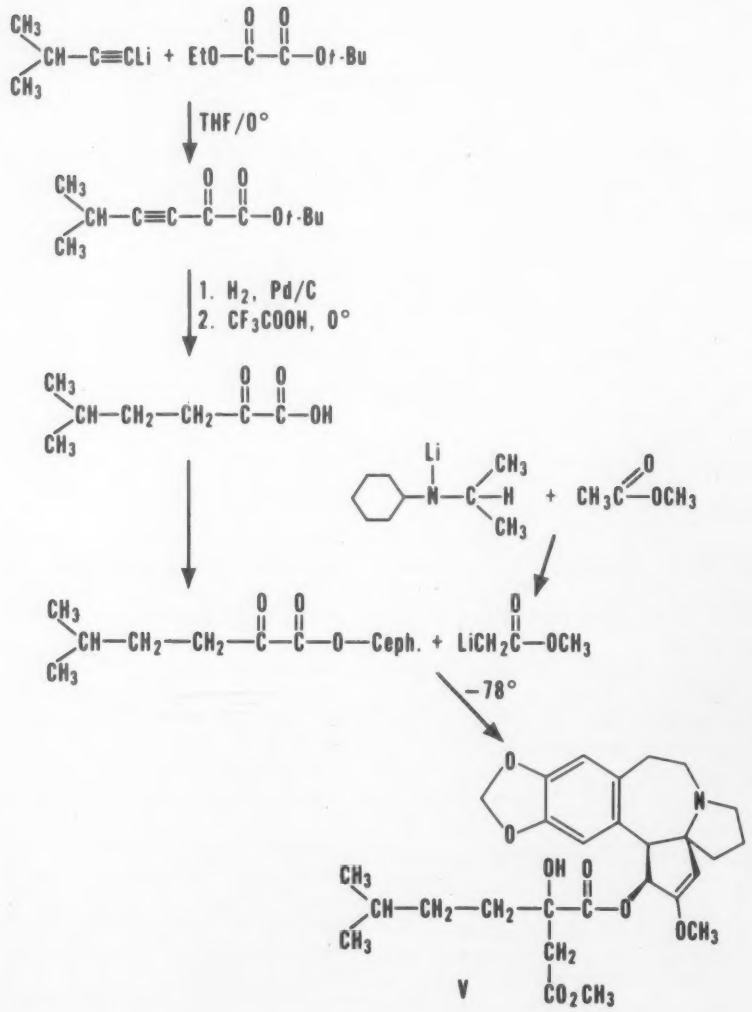


FIGURE 7

alkaloid,[3] which was converted to cephalotaxine in a two-step reaction.

Spirocycle XIX (fig 9) was a key intermediate in the total synthesis carried out by Semmelhack et al (19). They constructed XIX from 2-ethoxy-1-pyrroline (XVII); two three-carbon side chains were attached to XVII by consecutive reactions with allyl magnesium bromide. After ozonolysis and other intermediate transformations, these side chains were cyclized by an acyloin condensation and the resulting product was methylated to give XIX. Another important intermediate was provided by reducing β-chloropiperonylacetic acid to an alcohol which was converted to its *p*-nitrobenzenesulfonate ester (XX). Intermediates XIX and XX were condensed to give tertiary amine XXI; cyclization was then possible via a nucleophilic addition on a benzyne intermediate to give cephalotaxinone (XXII), a compound which can be reduced to cephalotaxine in a single step. In a subsequent refinement of this approach, the final ring closure was achieved by a nucleophilic aromatic substitution mediated by bis(1,5-cyclooctadiene) nickel (19).

Dolby and co-workers (18) undertook a synthesis of cephalotaxine by a route similar to that of Weinreb and Auerbach (17). However, their approach was

thwarted by unwanted rearrangements after condensing bromoacetone with enamine XIV (fig 8). This rearrangement was explained in terms of a mechanism involving ten-membered ring intermediates; similar macrocyclic intermediates may be involved in the unexpected racemization that occurs during formation of cephalotaxine methiodide (7).

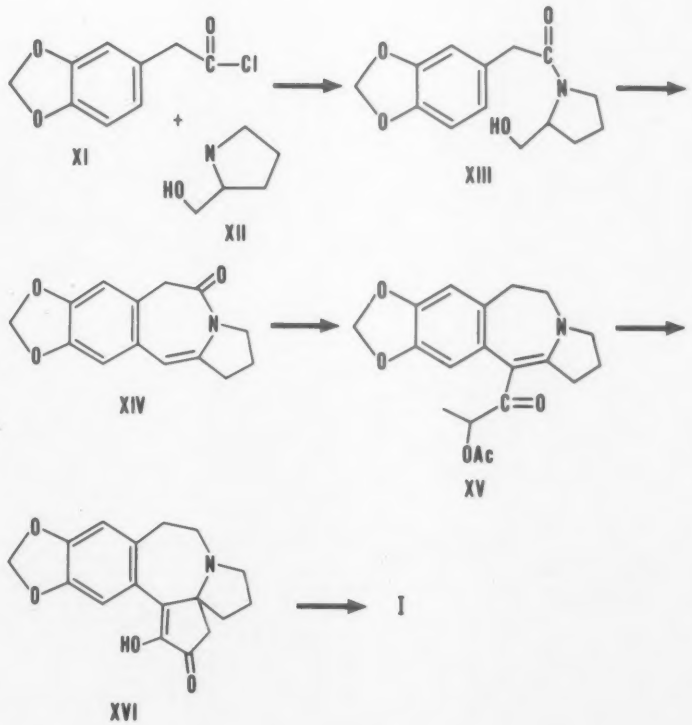


FIGURE 8

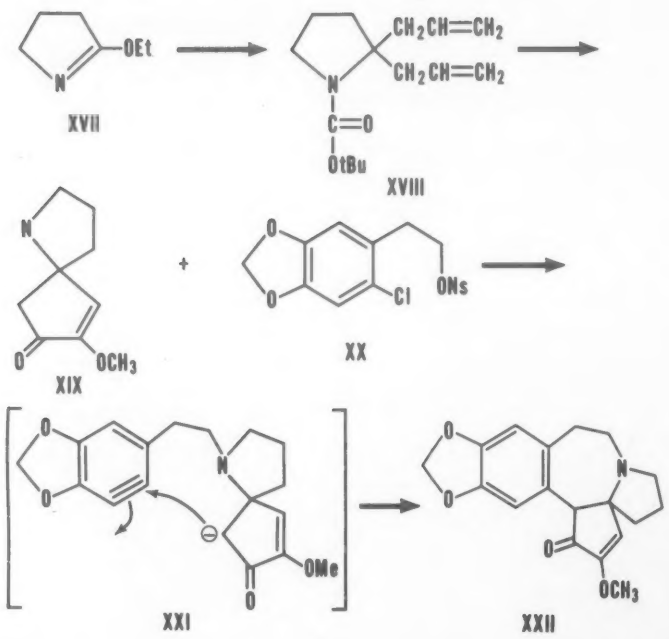


FIGURE 9

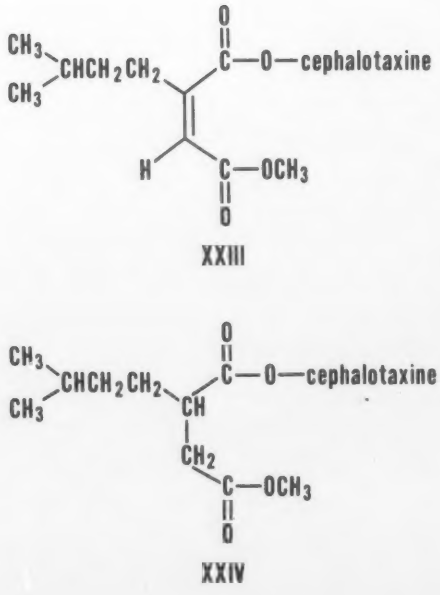


FIGURE 10

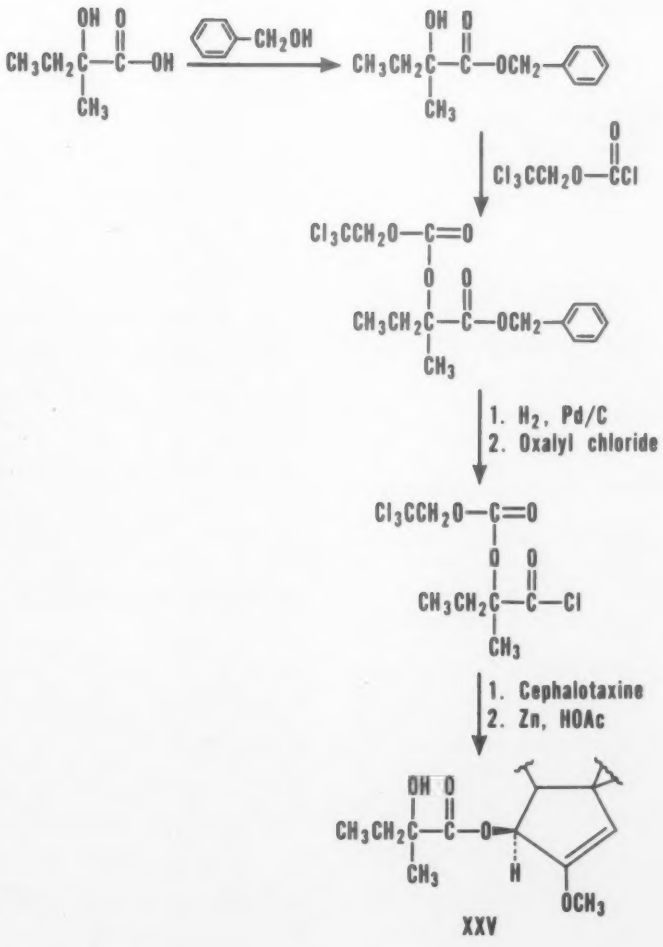


FIGURE 11

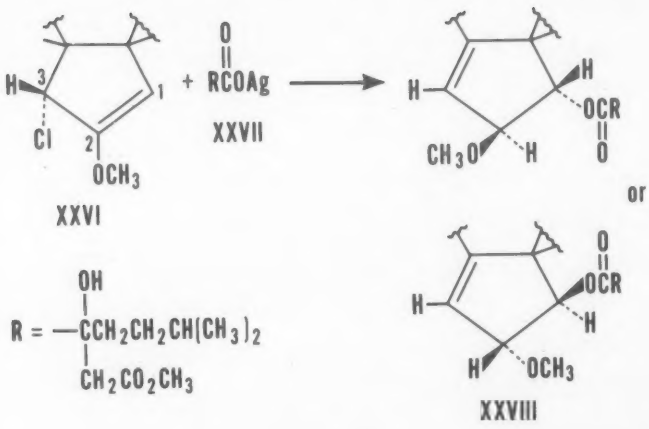


FIGURE 12

Structure-Activity Relationships Among Esters of Cephalotaxine

As noted above, cephalotaxine per se is inactive and its ester congeners differ somewhat in their antitumor activity. The effects of some other structural variations in the acyl moiety have been examined. Cephalotaxine acetate is inactive and so are two esters (fig 10, XXIII and XXIV) which are closely related to deoxyharringtonine but lack the hydroxyl function in the side chain. The 2-hydroxy-2-methylbutyrate ester (fig 11, XXV) of cephalotaxine was prepared because of its steric resemblance to harringtonine, homoharringtonine, and deoxyharringtonine; however, XXV proved to be inactive (20). A "rearranged" ester of cephalotaxine (fig 12, XXVIII), isomeric with deoxyharringtonine (VI), was prepared for biologic evaluation (21). Treatment of cephalotaxine with thionyl chloride provided epicephalotaxyl chloride (XXVI) and when XXVI was treated with the silver salt (XXVII) of the requisite half ester, XXVIII was formed stereospecifically. Unfortunately, XXVIII likewise was found to be devoid of activity. Recently, we have succeeded in preparing active esters of cephalotaxine with "unnatural" acyl groups (40). Little "rhyme or reason" has emerged to provide a basis for rationalizing the structure-activity relationships of cephalotaxine esters.

Companion Alkaloids of Cephalotaxine

In addition to harringtonine and related active esters (II–V), cephalotaxine has several other companion alkaloids that have been isolated from various *Cephalotaxus* species. 11-Hydroxycephalotaxine (fig 13, XXIX) and the related ketal drupacine (fig 13, XXX) occur in *C. drupacea* Sieb. and Zucc. (*C. harringtonia* var *drupacea* [Sieb. and Zucc.] Koidz.) (3,5,22,23). 11-Hydroxycephalotaxine can be con-

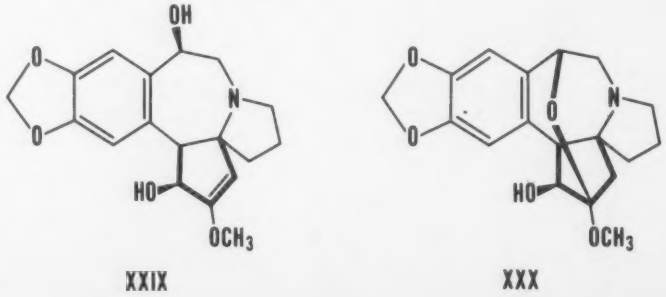
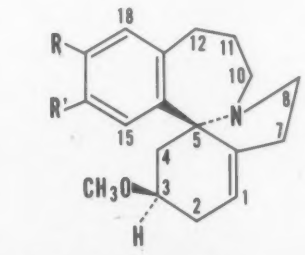


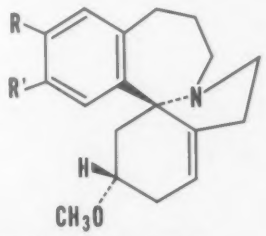
FIGURE 13

verted to drupacine under mild acidic conditions by addition of its C-11 hydroxyl function across the transannular double bond. This observation raised the suspicion that drupacine might be an artifact of isolation, but controlled experiments demonstrated that at least a portion of it must occur naturally (22).

In addition to cephalotaxine and its derivatives, a

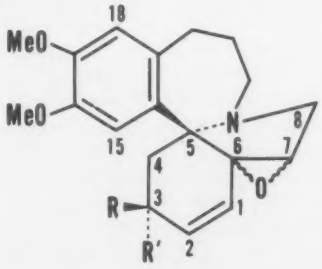


XXXI. R + R' = OCH_2O
XXXII. R + R' = OCH_3 + OH
XXXIII. R = R' = OCH_3

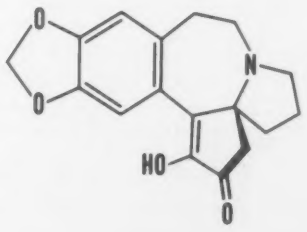


XXXIV. R + R' = OCH_2O
XXXV. R = OCH_3; R' = OH
XXXVI. R = R' = OCH_3

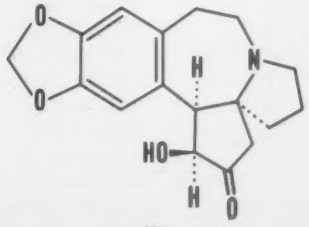
FIGURE 14



XXXVII. R = OMe; R' = H
XXXVIII. R = H; R' = OMe



XXXIX



XL

FIGURE 15

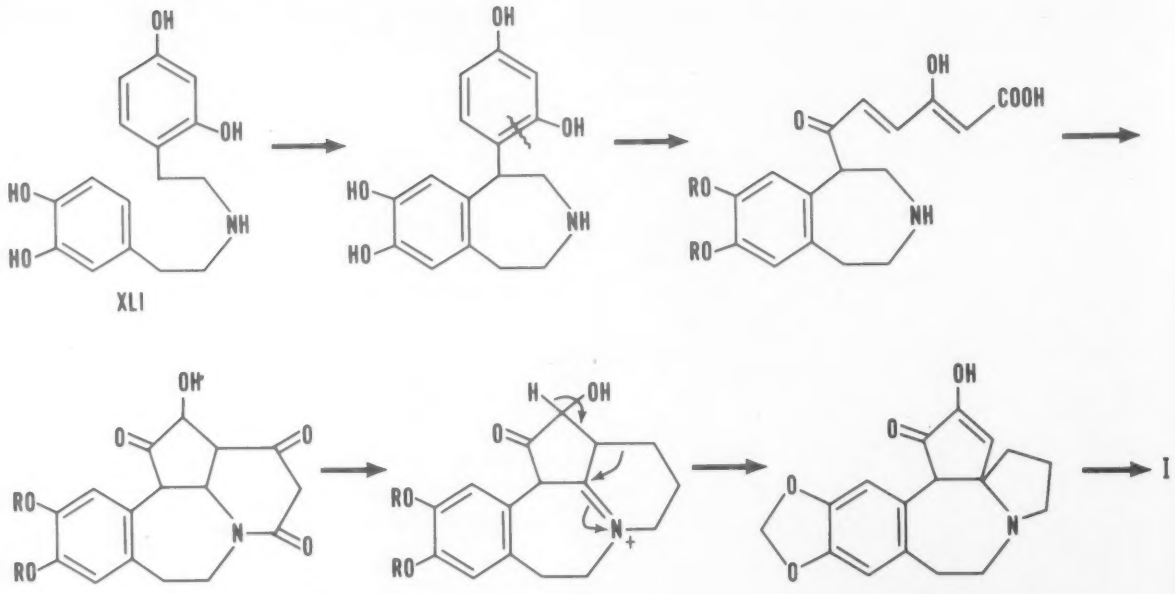


FIGURE 16

number of alkaloids of the homoerythrina series occur in *Cephalotaxus* species. Five derivatives (fig 14, XXXII–XXXVI) of schelhammericine (fig 14, XXXI) were isolated from *C. harringtonia* (24). *C. wilsoniana* Hayata occupies a unique position among *Cephalotaxus* species investigated since its alkaloids are comprised mainly of two members of the homoerythrina series—wilsonine (fig 15, XXXVII) and epiwilsonine (fig 15, XXXVIII)—accompanied by only small amounts of cephalotaxine and its acetate (25). In addition to these homoerythrina alkaloids, a few additional alkaloids of the cephalotaxine group occur naturally. Desmethylcephalotaxinone (fig 15, XXXIX) was isolated and characterized independently by Asada (23) and by Powell and Mikolajczak (26). Cephalotaxinone (XXII) and acetylcephalotaxine have been isolated in small amounts (24,25,27). Paudler and McKay (27) reported the natural occurrence of demethylcephalotaxine (fig 15, XL) and epicephalotaxine.

A recently developed gas-liquid chromatographic procedure facilitates analysis of crude mixtures of *Cephalotaxus* alkaloids (28). Table 3 summarizes some representative results from various species and plant parts. These figures emphasize the ubiquitousness of cephalotaxine and the low concentration in which the two most active alkaloids, harringtonine and homoharringtonine, are generally found.

Biosynthesis of *Cephalotaxus* Alkaloids

The discovery that certain homoerythrina alkaloids are congeners of cephalotaxine and its derivatives gave rise to the proposal that these two groups are derived from common biogenetic precursors (24). Previous investigations had established 1-benzyl-1,2,3,4-tetrahydroisoquinoline, presumably derived from tyrosine, as the precursor of erythrina alkaloids in a sequence involving oxidative phenol coupling. Fitzgerald and co-workers (29) extended this concept to the homoerythrina series by postulating a 1-phenylethyl-1,2,3,4-tetrahydroisoquinoline precursor. By an appropriate change in ring closure, Fitzgerald's scheme was adapted to the *Cephalotaxus* series (24). However, the first experimental evidence pointed to biosynthesis of cephalotaxine from tyrosine in an altogether unexpected manner. Parry and Schwab (30) observed that [3-^{14}C]-DL-tyrosine is incorporated into cephalotaxine in such a way that all of the radioactive labeling resides at C-4 and C-11, both of which are attached to the aromatic ring, thus implicating an intermediate such as XLI (fig 16). On the basis of this experiment and others in which [1-^{14}C]- or [2-^{14}C]-tyrosine was fed to *Cephalotaxus* plants, Parry and Schwab tentatively postulated the biosynthetic sequence outlined in figure 16.

TABLE 3.—Gas-liquid chromatographic analyses of selected *Cephalotaxus* alkaloid extracts

Species, plant part	Percentage of total alkaloids—							
	I	II	III	IV	V	XXIX	XXX	XXXVII + XXXVIII
C. harringtonia (Forbes) K. Koch—								
Var. *drupacea* (Sieb. & Zucc.) Koidz. seed	33	8.3	3.9	1.2	0.7	9.6	35	6.5
Var. *harringtonia* root	39	6.6	19	7.5	2.4	1.1	3.7	3.4
Var. *harringtonia* leaf	32	2.4	13	4.1	1.3	2.6	5.3	2.9
Var. *harringtonia* whole plant	45	3.7	20	8.5	1.5	2.1	3.7	2.2
C. fortunei Hook.—								
Leaf	64	0.3	0.3	0.1	—	1.4	6.8	4.2
Seed	52	4.4	0.9	trace	0.4	2.2	7.0	4.6
C. wilsoniana Hayata seed	29	6.1	0.4	0.2	—	0.9	0.7	45
C. griffithii Hook. root and leaf	40	0.9	11	1.5	4.3	2.5	9.0	1.7

Mechanism of Physiologic Action of Harringtonine and Related Alkaloids

Some research has been done which throws light on the mechanism by which harringtonine and related alkaloids exert their antitumor activity. Grollman and Huang (31,41) as well as Tscherne and Pestka (32) have shown that these compounds are inhibitors of protein synthesis in cell culture systems; reactions mediated by RNA are inhibited.

Addendum

Since this manuscript was originally submitted, there have been further significant developments related to *Cephalotaxus* alkaloids. Arora et al (13) have extended and confirmed their earlier X-ray crystallographic work on cephalotaxine *p*-bromobenzoate by comparable studies on underivatized cephalotaxine (33). The investigations of Brandänge et al (15), in which the acyl groups of harringtonine and homoharringtonine were shown to have the *R* configuration, have been extended through chemical synthesis by Spencer et al (34). Two groups of Chinese chemists (35, 36) have reported indirect conversions of cephalotaxine to one or more of its active esters. In connection with their biosynthetic studies, Schwab et al (37) converted cephalotaxine to a seco-dienone (related to macrocycles of Weinreb and Auerbach [17] and Dolby et al [18]) and other novel derivatives. McDonald and Suksamrarn (38) have synthesized certain compounds related to homoerythrina alkaloids and also to cephalotaxine; this work possibly may open the way for a biomimetic total synthesis of cephalotaxine. After further experimentation, Parry and Schwab (personal communication) have some reservations about the implications of their previously reported biosynthetic investigation of cephalotaxine (30). Battersby and co-workers (39) reported studies of the biosynthesis of a homoerythrina alkaloid, schelhammeridine, with preliminary results compatible with the phenol-coupling concept.

REFERENCES

1. PERDUE RE, SPETZMAN LA, and POWELL RG. *Cephalotaxus*—source of harringtonine, a promising new anti-cancer alkaloid. Am Hort Mag 49:12–22, 1970.
2. POWELL RG, ROGOVIN SP, and SMITH CR. Isolation of antitumor alkaloids from *Cephalotaxus harringtonia*. Ind Eng Chem Prod Res Develop 13:129–132, 1974.
3. POWELL RG, WEISLEDER D, and SMITH CR. Antitumor alkaloids from *Cephalotaxus harringtonia:* structure and activity. J Pharm Sci 61:1227–1230, 1972.
4. PAUDLER WW, KERLEY GI, and MCKAY J. Alkaloids of *Cephalotaxus drupacea* and *Cephalotaxus fortunei*. J Org Chem 28:2194–2197, 1963.
5. MCKAY J. A structural investigation of the alkaloids of *Cephalotaxus drupacea*. PhD dissertation, Ohio University, 1966.
6. POWELL RG, WEISLEDER D, SMITH CR, ET AL. Structure of cephalotaxine and related alkaloids. Tetrahedron Lett, pp 4081–4084, 1969.
7. ABRAHAM DJ, ROSENSTEIN RD, and MCGANDY EL. Structure of cephalotaxine methiodide. Tetrahedron Lett, pp 4085–4086, 1969.
8. POWELL RG, WEISLEDER D, SMITH CR, ET AL. Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahedron Lett, pp 815–818, 1970.
9. MIKOLAJCZAK KL, POWELL RG, and SMITH CR. Deoxyharringtonine, a new antitumor alkaloid from *Cephalotaxus:* structure and synthetic studies. Tetrahedron 28:1995–2001, 1972.
10. AUERBACH J, IPAKTCHI T, and WEINREB SM. Synthesis of the diacid sidechain of deoxyharringtonine. Tetrahedron Lett, pp 4561–4564, 1973.
11. IPAKTCHI T, and WEINREB SM. Relative configuration of the diacid sidechain of isoharringtonine. Tetrahedron Lett, pp 3895–3898, 1973.
12. KELLY TR, MCKENNA JC, and CHRISTENSON PA. Regiospecific synthesis of the acyl portion of harringtonine. Tetrahedron Lett, pp 3501–3504, 1973.
13. ARORA SK, BATES RB, GRADY RA, ET AL. Crystal and molecular structure of cephalotaxine *p*-bromobenzoate. J Org Chem 39:1269–1271, 1974.
14. BRANDÄNGE S, JOSEPHSON S, VALLÉN S, ET AL. Absolute

configuration of 2,3-dihydroxy-2-isopentylbutanedioic acid, a component of the alkaloid isoharringtonine. Acta Chem Scand B28:1237–1238, 1974.
15. BRANDÄNGE S, JOSEPHSON S, and VALLÉN S. Absolute configurations of 2-alkylmalic acids. Acta Chem Scand B28:153–156, 1974.
16. MIKOLAJCZAK KL, SMITH CR, JR, WEISLEDER D, ET AL. Synthesis of deoxyharringtonine. Tetrahedron Lett, pp 283–286, 1974.
17. WEINREB SM, and AUERBACH J. Total synthesis of *Cephalotaxus* alkaloids. J Am Chem Soc 97:2503–2506, 1975.
18. DOLBY LJ, NELSON SJ, and SENKOVICH D. Studies of the synthesis of cephalotaxine. J Org Chem 37:3691–3695, 1972.
19. SEMMELHACK MF, CHONG BP, STAUFFER RD, ET AL. Total synthesis of *Cephalotaxus* alkaloids. J Am Chem Soc 97:2507–2516, 1975.
20. MIKOLAJCZAK KL, SMITH CR, and POWELL RG. Partial synthesis of harringtonine analogs. J Pharm Sci 63:1280–1283, 1974.
21. MIKOLAJCZAK KL, POWELL RG, and SMITH CR, JR. Preparation and antitumor activity of a rearranged ester of cephalotaxine. J Med Chem 18:63–66, 1975.
22. POWELL RG, MADRIGAL RV, SMITH CR, JR, ET AL. Alkaloids of *Cephalotaxus harringtonia* var. *drupacea*. 11-Hydroxycephalotaxine and drupacine. J Org Chem 39:676–680, 1974.
23. ASADA S. Alkaloids isolated from *Cephalotaxus drupacea* Sieb. et Zucc. J Pharm Soc Jap 93:916–924, 1973.
24. POWELL RG. Structures of homoerythrina alkaloids from *Cephalotaxus harringtonia*. Phytochemistry 11:1467–1472, 1972.
25. POWELL RG, MIKOLAJCZAK KL, WEISLEDER D, ET AL. Alkaloids of *Cephalotaxus wilsoniana*. Phytochemistry 11:3317–3320, 1972.
26. POWELL RG, and MIKOLAJCZAK KL. Desmethylcephalotaxinone and its correlation with cephalotaxine. Phytochemistry 12:2987–2991, 1973.
27. PAUDLER WW, and MCKAY J. The structures of some of the minor alkaloids of *Cephalotaxus fortunei*. J Org Chem 38:2110–2112, 1973.
28. SPENCER GF, PLATTNER RD, and POWELL RG. Quantitative Gas Chromatography and Gas Chromatography-Mass Spectrometry of Cephalotaxus Alkaloids. J Chromatog 120:335–341, 1976.
29. FITZGERALD JS, JOHNS SR, LAMBERTON JA, ET AL. Alkaloids of *Schelhammera pedunculata* (Liliaceae). Aust J Chem 22:2187–2201, 1969.
30. PARRY RJ, and SCHWAB JM. Biosynthesis of *Cephalotaxus* alkaloids. I. J Am Chem Soc 97:2555–2556, 1975.
31. GROLLMAN AP, and HUANG MT. Inhibitors of protein synthesis in eukaryotes: tools in cell research. Fed Proc 32:1673–1678, 1973.
32. TSCHERNE JS, and PESTKA S. Inhibition of protein synthesis in intact HeLa cells. Antimicrob Agents Chemother 8:479–487, 1975.
33. ARORA SK, BATES RB, GRADY RA, ET AL. Crystal and molecular structure of cephalotaxine. J Org Chem 41:551–554, 1976.
34. SPENCER HK, KHATRI HN, and HILL RK. Stereochemistry of some acetyl coenzyme A condensations. Bioorganic Chem 5:177–186, 1976.
35. LI S-W, and DAI J-Y. A study on deoxyharringtonine and its analogs. Hua Hsueh Hsueh Pao 33:75–78, 1975.
36. ANON. Partial synthesis of harringtonine. K'o Hsueh T'ung Pao 20:437, 1975.
37. SCHWAB JM, PARRY RJ, and FOXMAN BM. Unusual addition reactions of seco-cephalotaxine. J Chem Soc (Chem Commun), 1975, pp 906–907.
38. MCDONALD E, and SUKSAMRARN A. Total synthesis of compounds related to the homo-erythrina alkaloids. Tetrahedron Lett, 1975, pp 4425–4428.
39. BATTERSBY AR, MCDONALD E, MILNER JA, ET AL. Biosynthesis of schelhammeridine: mode of specific incorporation of [2-^{14}C]tyrosine. Tetrahedron Lett, 1975, pp 3419–3422.
40. MIKOLAJCZAK KL, SMITH CR, and WEISLEDER D. Synthesis of cephalotaxine esters and correlation of their structures with antitumor activity. J Med Chem. In press.
41. HUANG M-T. Harringtonine, an inhibitor of protein synthesis. Mol Pharmacol 11:511–519, 1975.

Oncogenic and Tumor-Promoting Spermatophytes and Pteridophytes and Their Active Principles

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SUMMARY

A survey and discussion are presented of plants classified as Spermatophyta and Pteridophyta, extracts of which have been shown to be oncogenic or tumor-promoting in animals. The active oncogenic and tumor-promoting principles, where known, have been identified. They represent tannins; pyrrolizidine, indole, tropolone, quinoline, purine, and benzophenanthridine alkaloids; nitroso compounds; triterpene glycosides; lignans; isoflavans; allyl benzenoids; simple α-pyrenes; and carbocyclic hydroxy acids. A total of 28 compounds of known structure have been identified as oncogens and several phorbol esters as tumor-promoters. Plants known to contain any of the 28 oncogens (excluding shikimic acid and caffeine) have been tabulated; they represent at least 454 species, 110 genera, and 34 families of Spermatophyta and Pteridophyta.

[Cancer Treat Rep 60:1171–1214, 1976]

Plants, including those used as foods by man, are potential sources of oncogenic substances of probably greater importance than has been previously recognized. Our knowledge of the extent to which known plant oncogens are distributed in nature is limited, and the identity of the active principles in plants whose extracts are known to be oncogenic for animals is even less well documented.

There are two major reasons for studying the oncogenic activity of plants. The first is to identify plants used as food and/or drugs by man and as food for animals that could be possible initiators of the oncogenic process. Removal of such plants from the diet could conceivably reduce the incidence of human cancer.

Loss of livestock grazing on oncogenic plants is a major problem in certain areas of the world. In the US during the period 1955–1962, malignant tumors were found in only 0.227% of the cattle slaughtered under the supervision of the US Department of Agriculture (1). In contrast, it is estimated that the incidence of rumenal cancer in cattle in the Nasampolai Valley of Kenya is 2.5%. Although there is as yet no explanation for the high incidence of cancer in cattle of that area, it may be due to the plants on which they graze.

There is a second reason for studying oncogenic plants. Once the chemical nature of the oncogenic principle(s) is(are) identified, detailed studies of the mechanism by which such agents induce oncogenesis can be undertaken. The results could be of enormous benefit in our understanding of the etiology of cancer.

To the best of our knowledge, the area of oncogenic plants has never been reviewed as a whole. However, there are excellent reviews on the tumor-promoters of croton oil (2–5) and other members of the Euphorbiaceae, especially *Euphorbia* species (3–5), as well as on the oncogenic activity of pyrrolizidine alkaloids (6) and of cycasin and the cycads (7–11); a few limited reviews on specific higher plant oncogens (4,12) have also been published.

This paper will be restricted to a consideration of plants classified as Spermatophyta (Angiospermae and Gymnospermae) and Pteridophyta that have been shown to induce tumors in man and/or in animals, including the active principles from these

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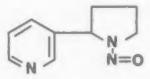
plants where known. Fungal and animal-derived oncogens and tumor-promoters will not be treated.

Tobacco has been intentionally omitted from this review, since the oncogens present are presumably formed during combustion and may not be naturally occurring. On the other hand, a very recent report has revealed that *N'*-nitrosonornicotine (I), a potential oncogen, has been positively identified in unburned tobacco in concentrations ranging from 1.9 to 88.6 parts per million (13). This is the highest value yet reported for an environmental nitrosamine. Other nitrosamines reported to be present in tobacco, ie, *N,N*-dimethylnitrosamine and *N*-methyl-*N*-ethylnitrosamine (14), have been found only in smoke condensates and were undoubtedly formed during combustion.



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The literature covered in this review is comprehensive, as far as possible, through April 1976.

METHODS OF TESTING FOR ONCOGENIC AND TUMOR-PROMOTING ACTIVITY

A few brief comments should be made concerning the laboratory methods used in testing for the oncogenic or tumor-promoting activity of plants, plant extracts, and their active principles. The oral and cutaneous routes of administration have been the most widely used in such tests; indeed, the cutaneous route (also known as skin "painting" [15]) is the method of choice in testing for tumor-promoting activity (16). For oral administration, the test substance may be mixed in the diet or in the drinking water, or may be administered by gavage or in a capsule (17). Cutaneous administration formerly involved using a small brush to "paint" a solution of the test material on the shaved skin of an animal (15), but applying measured volumes by means of a pipette is now thought to be more suitable (18). Other means by which the test materials may be administered include various types of parenteral injections, inhalation, and implantation into the subcutaneous tissue or the bladder (15). It is not clear, however, if tumors formed in implantation studies are due to oncogenicity of the test compounds, chronic irritation from the foreign body, and/or a "promoting" (cocarcinogenic [5]) action of the implant itself (17,19). A compound may produce tumors locally or at a distant site when administered by one route but not when administered by another (17).

The selection of species to be used in oncogenesis studies is based largely on the necessity of having to continue such tests for most or all of the lifespan of the animals (16); rats and mice, therefore, are the species usually used. It should be pointed out, nevertheless, that all species do not respond similarly to all oncogenic agents (17). Dosing at at least two levels is begun in both males and females when they are 5–8 weeks of age (17); differences in response between the sexes are often seen. Use of newborn mice has been advocated because of their greater sensitivity to many oncogens (17). The animals are killed for histopathologic study after at least 2 years for rats and 18–20 months for mice (17); such study is necessary since tumors may arise in various organs following systemic administration of an oncogen and even after cutaneous administration if the compound is absorbed from and/or licked off the application site. The latter possibility is lessened if the vehicle used is a volatile solvent, which facilitates penetration of the material into the skin (18). In order that test results be as unambiguous as possible, a sufficient number of control animals are observed and killed for histopathologic study (17); equally important is the selection of a strain that has a low spontaneous tumor incidence. Positive controls are also necessary (17) in order to demonstrate that the strain being used is sensitive to known oncogens.

The classic rodent lifetime feeding test for oncogenesis tends to predict well for man but it is time-consuming (20). Consequently, a number of short-term, preliminary tests have been devised (17). Perhaps the most interesting are the in vitro mutagenesis assays, positive results in which indicate that the test compound can alter information transferred through DNA (20); it has been postulated that such an alteration may be the ultimate mechanism of oncogenesis. Indeed, the "active forms" of a large number of known oncogens are mutagenic (21,22). The phrase "active form" is important because it points up a drawback to some of these in vitro assays. Oncogens that cause tumors at the point of application are presumed to be active per se (18); others, however, require enzymatic or nonenzymatic transformation in order to become active oncogens. Bacteria, unfortunately, do not usually resemble mammals in their ability to activate oncogens (21); this problem may be partially solved by incubating a liver homogenate together with the test compound and the bacterial test system.

Some substances, known as tumor-promoting agents (cocarcinogens [5]), are themselves non-oncogenic or at most only weakly so, the best example being croton oil and the phorbol esters that it contains (23). Such agents elicit oncogenic activity

from compounds with only marginal or no activity alone (15). Adequate testing of compounds for oncogenicity, therefore, includes assays employing a known promoting agent (18); conversely, testing for promoting, or cocarcinogenic, activity involves employing the test compound with a subthreshold dose of a known oncogen (5,15). A weak oncogen, or a subthreshold dose of a potent oncogen, applied to the skin of mice, for example, produces an unidentifiable effect known as initiation, a latent potentiality for malignant growth (15,18); subsequent, repeated applications of the promoting agent result in the development of perceptible tumors.

At least one series of experiments suggests that bile acids may act as promoting agents (24). An increase in bile acid content in the intestines of rats, produced either by the ingestion of cholestyramine or by mechanical means, was associated with an enhanced oncogenic effect of several intestinal oncogens; a similar result was obtained in the presence of a high beef fat diet which might also have increased intestinal bile acid content.

Finally, not all tumors produced in experimental studies of potential oncogens and tumor-promoters are malignant; many are benign. Consequently, the more general terms oncogenic and tumor-promoting may be considered preferable to the terms carcinogenic and cocarcinogenic respectively, since the latter two imply malignancy.

ONCOGENIC PTERIDOPHYTA

Bracken Fern as an Oncogenic Agent

The bracken fern, *Pteridium aquilinum* (L.)Kuhn (*Pteris aquilina*) (Polypodiaceae), is present throughout the northern hemisphere. This plant is toxic to animals and is a particular problem for grazing cattle (25–34). Its major toxic manifestation following ingestion by cattle is chronic enzootic hematuria (35–39). The bracken fern causes tumors of the urinary bladder as well as tumors at other sites in animals (36–58). Feeding studies demonstrated that bracken fern induces tumors in the urinary bladder of cows (42,47,54) and mice (49), the intestine of rats (44–47,51,55,56,58) and sheep (59), the mouse lung and guinea pig bladder (27,49,56), and the Japanese quail (57). Urine extracts from bracken-fed cattle, and from cows bearing "spontaneous" urinary bladder tumors, contained oncogenic substances that subsequently induced tumors in the bladders of calves, dogs, rats, and mice, and in the skin of mice (37–39, 48).

Bracken fern can produce tumors at various sites in the same species of animal, and tumors are produced in both males and females. Feeding experiments in rats have shown that all parts of the bracken fern are oncogenic (58). However, the latent period of tumor induction was found to be shorter, and the multiplicity of tumors higher, with the rhizome than with the fronds (58). The oncogenic activity of the young curled tops of bracken was found to be greater than that of the stalks and that of the rhizomes was greater than that of the young fronds (58).

In 1970 Pamukcu and co-workers (49) suggested that young bracken fronds ("fiddle heads"), used as a human food in the US, New Zealand, and especially Japan, might contribute to the high incidence of stomach cancer in these countries. In 1972, Hirono and co-workers (51) reported data (*see* table 1) that supported this hypothesis. They administered unprocessed bracken fern to one group (I) of rats and processed bracken fern to four other groups (II, III, IV-1, and IV-2) of rats. For 4 months, groups II and III were given pellets containing processed bracken treated with boiling water containing wood ash or sodium bicarbonate; when used as a food, young bracken is immersed in boiling water containing wood ash or sodium bicarbonate to remove the "harshness." Groups IV-1 and IV-2 received pellets for 4 or 9 months respectively; these pellets contained salted bracken, since one form of human use of bracken is to store it with salt prior to immersion in boiling water. An additional group of rats (IV-3) received pellets of basal diet with sodium chloride added for 9 months. A control group of rats received a normal diet. The results of these studies are summarized in table 1.

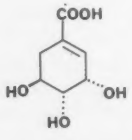
Table 1. —Tumor induction in rats fed bracken fern (51)

Group	Preparation of bracken fern	Mos of feeding	Rats with tumors/rats surviving after 7 mos
I	Unprocessed	4	10/12
II	Processed with boiling water + wood ash	4	3/12
III	Processed with boiling water + sodium bicarbonate	4	1/10
IV-1	Salted bracken	4	1/10
IV-2	Salted bracken	9	1/11
IV-3	Basal diet + sodium chloride	9	0/15
Control	normal diet	16	0/12

These studies clearly demonstrated that the various methods of preparing "fiddle heads" from bracken fern for human consumption removed most, but not all, of the oncogenic material. Thus, bracken fern could well be a contributing factor in human cancer.

In 1971, Leach and co-workers (53) isolated a crystalline material claimed to be the oncogenic principle of bracken. The substance had a melting point of 110°C and the molecular formula was $C_7H_8O_4$ (molecular weight, 156). It was very soluble in water but less so in methanol, was unstable in aqueous solution, and was polymerized when heated in acidic solution. Addition of alkali abolished the oncogenic activity. The nuclear magnetic resonance spectrum for the compound revealed only the presence of $-CH_2$, $-COOH$, and $-CH_3$ protons, while the infrared spectrum displayed bands characteristic for $-OH$, $C=O$, and alkyl groups. The ultraviolet spectrum displayed only end absorption.

In 1974, Evans and Osman (59) succeeded in identifying the oncogenic component of *Pteridium aquilinum* as shikimic acid (II). Shikimic acid given ip at doses of 1–20 mg/animal or orally at 100 mg/animal induced malignancies in mice (59). This finding is of great potential significance, since shikimic acid is a ubiquitous component of plants. Thus, a recent survey of 83 species of Angiosperm leaf samples from Japan revealed that 43 species contained concentrations of shikimic acid ranging from 0.001% to 0.193% fresh weight. An additional 13 species contained trace amounts of shikimic acid, ie, < 0.001%, whereas only 27 of the 83 species were devoid of this substance (60). Further, it was shown that the average concentration of shikimic acid was 0.004% in the leaves of monocots, 0.006% in herbaceous dicots, and 0.016% in woody dicots (60).



II

ONCOGENIC GYMNOSPERMAE

Cycads as Sources of Oncogens

Currently there are believed to be about 100 species of cycads distributed in nine genera, ie, *Bowenia, Ceratozamia, Cycas, Dioon, Encephalartos, Microcycas, Macrozamia, Stangeria,* and *Zamia.* All of these species are indigenous to the tropics or subtropics, where their toxic effects in man and animals have been known for many years. The major toxic effects produced by these plants are gastrointestinal and neurologic in nature. Dr. Marjorie Whiting, who must be given the major credit for bringing attention to the problem of cycad toxicity, reviewed the literature on this subject up to 1967 (7–9). The literature review was updated in 1969 (10) and in 1972 (11). Conference proceedings of meetings on the subject of cycad toxicity were published in 1964 (61), 1965 (62), and 1967 (8).

Although no reports exist attributing cancer in humans to the ingestion of cycads, a potent oncogen has been found in these plants. Natives using cycads for food normally do not experience toxic effects, presumably since the method of preparation removes the toxic material. Efforts began in the 1870s to isolate the toxic principle(s) from cycads; however, it was not until 1941, when Cooper (63) reported the isolation of macrozamin (III) from *Macrozamia spiralis* (Salisb.)Miq., that a toxic constituent was identified in this group of plants.

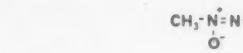
Since that time a number of related compounds have been isolated from various cycads: cycasin (IV) (64–66); neocycasin A (V) (67), B (VI) (68), C (VII) (69), D (70), E (VIII) (70,71), F and G (70); and the aglycone methylazoxymethanol (MAM) (IX) (72). The structures of these compounds, where known, are presented in figure 1.

The first study in which *Cycas circinalis* L. seed was shown to produce tumors in animals was published in 1963 (73). Additional studies demonstrated that the seed meal and husk were also oncogenic (74–77) in rats and guinea pigs, inducing tumors in the liver and/or kidneys.

Although the oncogenic agent in *Cycas circinalis* had not been definitely identified, Laqueur and his co-workers (73) suggested that it might be a metabolic breakdown product of cycasin (IV), a glycoside isolated from the cycads. The results of most subsequent work tend to support this suggestion. Cycasin administered orally was oncogenic in conventional but not germ-free rats (78–81); however, the aglycone of cycasin, MAM (IX), did produce tumors in germ-free rats when administered orally. It was concluded, therefore, that the proximate oncogen was MAM, liberated from the glycoside by the action of bacterial β-glucosidase in the intestinal tract (78–82).

Similar conclusions were reached concerning the toxicity of these compounds when the effects of ip versus oral administration were compared. Ip injections of cycasin were nontoxic to rats whereas oral administration of this compound was toxic (83,84); on the other hand, ip administration of the aglycone MAM was toxic.

Nevertheless, some investigators have found that



III-IX

Cycad constituent	Structure	R	Ref.
Macrozamin	III	β-D-primverosyloxy-	62,67
Cycasin	IV	β-D-glucosyloxy-	64-66
Neocycasin A	V	β-laminaribiosyloxy-	67
Neocycasin B	VI	β-gentiobiosyloxy-	68
Neocycasin C	VII	β-laminaritetraoxyloxy-	69
Neocycasin D	-	-	70
Neocycasin E	VIII	β-cellobiosyloxy-	70-71
Neocycasin F	-	-	70
Neocycasin G	-	-	70
Methylazoxymethanol (MAM)	IX	H	72

FIGURE 1.—Structure of cycad oncogens.

sc administration of cycasin to newborn mice (85,86), newborn hamsters (87), and newborn rats (88) resulted in tumor formation; it may be of some significance that β-D-glucosidase has been found in the skin of newborn rats (89). A number of other papers reporting similar results have been published concerning the oncogenicity (90–94) and toxicity (90,94–103) of cycasin as well as the oncogenicity (90,104–106) and toxicity (90,101,106) of MAM. Cycasin has also been shown to have activity against some animal tumors (108).

ONCOGENIC ANGIOSPERMAE

Betel Nut (*Areca catechu* L., Palmae)

Although some investigators believe that there is no relationship between betel chewing and oral cancer (109–114), at least an equivalent number of investigators have concluded that such a relationship does exist (110,114–122). However, in most of the latter studies, the betel quid also contained materials such as *Piper betle* L., *Acacia catechu* (L.fil.)Willd., *A. suma* (Roxb.)Buch.-Ham ex Voigt, *Uncaria gambir* (Hunter)Roxb., *Curcuma aromatica* Salisb., tobacco (*Nicotiana tabacum* L.), cardamom, clove, anise, and/or lime (123) in addition to betel nut. Indeed, Davis (124) claims that the oncogen in betel quid is lime.

Since the method of preparing betel quid varies in different geographic areas, it is very difficult to determine the actual contribution, if any, of the various constituents in the quid to the induction of oral cancer in man.

Laboratory studies on the oncogenicity of betel are also confusing. Woelfel and co-workers (125) tested the ether, alcohol, and unsaponifiable fractions of betel nuts in mice but failed to induce tumors. Dunham and Herrold (126) incorporated several betel quid ingredients separately into beeswax, inserted them into hamster buccal pouches, and observed the animals for up to 35 months. The ingredients studied were *Areca catechu* (betel), betel leaf (*Piper betle*), tobacco leaf (*Nicotiana tabacum*), gambir (*Uncaria gambir* vine), black pepper (*Piper nigrum* L. fruit), nutmeg (*Myristica fragrans* Houtt. seed), commercial Chinese incense, and beeswax as a control. Of 375 hamsters used, 19 developed pouch lesions, but these were not malignant. A number of hamsters developed malignant tumors in other parts of the body, but spontaneous development of tumors in hamsters is not unexpected (126). It was concluded that none of these materials induced tumors in the hamster cheek pouch under the conditions of the study (126).

Dimethyl sulfoxide (DMSO) extracts of betel nut, tobacco, and a combination of these were tested for oncogenic activity in the hamster buccal pouch (127). The betel nut extract induced leukoplakia in 19 of 21 hamsters and tumors in eight of 21 (table 2). A tobacco extract induced leukoplakia in 8 of 12 hamsters, but no tumors developed. A mixture of betel nut extract plus a tobacco extract induced leukoplakia in 18 of 21 hamsters and tumors in 16 of 21. Eleven DMSO-treated hamsters developed neither leukoplakia nor tumors. It was suggested that previous negative results might have been due to the insolubility of the oncogenic constituent(s) of betel, whereas in these experiments (127), the constituents had been solubilized in DMSO.

Table 2.—Incidence of leukoplakia and tumors in hamster buccal pouches after chronic topical application of DMSO extracts of betel nut and of a betel nut and tobacco mixture (127).

Treatment	No. animals	No. hamsters with leukoplakia	tumors
Tobacco	12	8	0
Betel nut	21	19	8
Betel nut + tobacco	21	18	16
DMSO	11	0	0

Wheat Germ Oil as a Potential Oncogen

Rowntree and co-workers (128–131) first reported the development of abdominal sarcomas in rats fed a crude ether extract of wheat germ oil. They reported negative results with wheat germ oil made

by the cold compression method (131) as well as with refined ether-extracted wheat germ oil (128,131) and benzene-extracted wheat germ oil (128); oils from other plants or animal sources similarly yielded negative results (128,131). Furthermore, the spontaneous tumor incidence in these workers' rats was reported to be very low (130,131). Subsequent studies by other groups, however, failed to confirm the tumorigenicity of ingested crude wheat germ oil (132–141). Even Rowntree was unable to duplicate his original results with orally administered crude ether-extracted wheat germ oil (142); tumors did develop, however, in a small number of rats administered such material ip. It is likely that the development of tumors in mice and rats following repeated sc or ip injections of ether-extracted wheat germ oil may represent a nonspecific reaction to chronic irritation (141); efforts to attribute the "activity" to a sediment in the crude oil have yielded equivocal results (141,142).

The reason for the discrepant results following oral administration of crude wheat germ oil remains unclear; that the positive results might have been due to an oncogenic agent arising as a result of the action of peroxides formed during extraction of the wheat germ with ether seems to have been ruled out (143).

Tannins as Oncogens

Geographic surveys of the relationship between oral cancer and plants suggest that oral cancer rates may be higher (144–152) in areas where herbal remedies are used extensively and/or plant materials are habitually chewed, ie, tobacco and betel nut. Morton (145–148), studying the relationship of esophageal cancer and medicinal plant usage especially in Curacao, suggested that tannins could be one of the plant constituents responsible for the induction of esophageal and other types of human cancer.

The observation that burn patients treated with tannic acid preparations often developed liver necrosis (153–155) prompted several workers to study this phenomenon in animals, and it was found that tannic acid was hepatotoxic (156–160). Shortly thereafter, a number of workers reported that tannic acid or tannin extracts from plants were oncogenic in animals (161–167). Further, tannic acid promotes the oncogenicity of 2-acetylaminofluorene (168).

Kirby has shown that the tannin-containing extracts from several plants (*see* table 3) produced either sarcomas or liver tumors in mice (167). It is interesting to note that extracts containing condensed tannins produced sarcomas at the site of injection, in addition to liver tumors. However, only liver tumors were produced by those extracts containing hydrolyzable tannins.

Studies on the oncogenic activity of several plants used extensively as herbal remedies in Curacao showed that extracts from nine of 21 species produced sarcomas in rats at the injection sites (169–172). These plants are listed in table 4. The oncogenic activity was further shown to be associated with the tannin fraction of *Krameria ixina* L., *K. triandra* Ruia and Pavon, and *Acacia villosa* (Sw.)Willd. (173).

Extracts of *Krameria ixina*, *Acacia villosa*, *Heliotropium ternatum* Vahl, and *Annona muricata* L. also produced a superficial spreading carcinoma of the hamster buccal-pouch epithelium following local administration (172). Tannins present in the first two of these plants are undoubtedly the oncogenic principles.

Recent evidence suggests that tannins and other polyphenolic plant constituents might play a role in oncogenesis by catalyzing *N*-nitrosamine formation

Table 3. — Induction of tumors in mice by tannin extracts (167)

Family	Plant	Common name	Sarcomas	Liver tumors
Anacardiaceae	*Schinopsis quebracho-colorado* (Schlecht.) Barkley & Mayer [*S. lorentzii* (Griseb.) Engl.]	Quebracho	8/10	5/10
Combretaceae	*Terminalia chebula* Retz.	Myrobalans	0/10	4/10
Fagaceae	*Castanea sativa* Mill.	Chestnut	0/10	4/10
Fagaceae	*Quercus aegilops* L.[a]	Valonea	0/10	1/10
Leguminosae	*Acacia pubescens* (Vent.) R.Br. [*A. mollissima* Willd.]	Mimosa	2/10	9/10
Myrtaceae	*Eucalyptus rediuca* Schauer	Myrtan	5/10	7/10
	Tannic acid	-	0/10	7/10

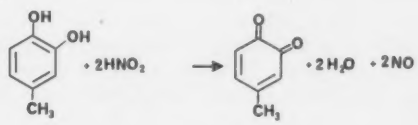
[a]The correctness of this binomial could not be verified in the current literature.

Table 4. —Oncogenic plants used in herbal remedies in Curacao (169-173)

Family	Plant	Part*	Activity in	Ref.
Annonaceae	*Annona muricata* L. "Sorsaka"	LF		166,168
Boraginaceae	*Heliotropium angiospermum* J.A.Murray "Cocolode"	PX		167
	Heliotropium ternatum Vahl "Sali"	LF		166,168
Krameriaceae	*Krameria ixina* L. "Cadia del Perro"	PX	Tannin fraction	170-173
	Krameria triandra Ruiz & Pavon	RT	Tannin fraction	173
Leguminosae	*Acacia villosa* (Sw.) Willd. "Watapana shimaron"	RT	Tannin fraction	171-173
	Gliricidia sepium (Jacq.) Kunth ex Steud. "Raton"	LF		166
Rutaceae	*Citrus aurantium* L.	LF		166
Sterculiaceae	*Melochia tomentosa* L.	RT		167

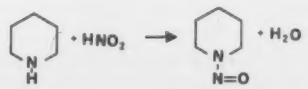
*LF, leaf; PX, aerial parts; RT, root.

from nitrite salts and secondary amines in the body. The oncogenic properties of secondary *N*-nitrosamines and related compounds is well established in animals (174,175), although their causal relationship to human cancer remains unclear. Indirect evidence for the catalytic role of *o*-phenolic compounds in forming *N*-nitroso derivatives has been demonstrated very recently by Challis and Bartlett (176). These investigators have shown that treatment of 4-methylcatechol (X) with dilute HNO_2 at pH 2–4 and 25°C yields the corresponding *o*-quinone (XI) with evolution of nitric oxide. The nitric oxide is then available for *N*-nitroso formation with appropriate amines. The more basic secondary amines, such as dimethylamine and piperidine (XII), react slowly with aqueous HNO_2 in the absence of added *o*-phenols (176). However, the addition of as little as



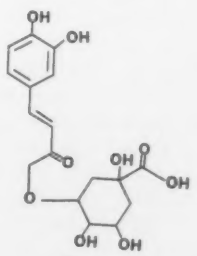
X XI

SCHEME 1.—Effect of nitrous acid on NO production by 4-methylcatechol.



XII XIII

SCHEME 2.—Action of nitrous acid on piperidine.



XIV

10^{-3} M 4-methylcatechol increases the rate of *N*-nitroso pyridine (XIII) formation by a factor of at least 1000 (176). Chlorogenic acid (XIV), a compound found in instant coffee at an approximate concentration of 13% by weight, similarly catalyzes *N*-nitrosamine formation (176); indeed *N*-nitrosamine formation was found to be catalyzed by the addition of "instant coffee" to buffered HNO_2-piperidine mixtures (176).

Whether such reactions occur in the body is not yet known. However, these studies of Challis and Bartlett (176) offer the first plausible explanation for the oncogenic effects of tannins and presumably other plant polyphenols.

Tea [*Camellia sinensis* (L.)Kuntze.] as a Potential Oncogen

Since common tea is rich in tannins, one might expect that excessive consumption of this beverage would result in an increased incidence of human cancer. In a recent epidemiologic study in selected

areas of Japan, data were collected associating the intake of tea-gruel (rice cooked with tea) with death rates from esophageal cancer (177). Animal studies (178,179) have shown that extracts from black tea are tumor-promoting when applied to mouse skin previously exposed to 4-benzopyrene. These findings suggest a link between tannin ingestion (especially in coffee and tea) and human cancer, but further studies are necessary to clarify this issue.

It is interesting to note that in addition to being oncogenic, many plant tannins have also been shown to have antitumor activity, chiefly against the Walker 256 carcinosarcoma (180–183), Sarcoma 45 (184), Sarcoma 180 (184), Guérin carcinoma (184), Ehrlich carcinoma (184), and Jensen sarcoma (185).

Pyrrolizidine Alkaloids as Oncogens

Many plants containing pyrrolizidine alkaloids are hazardous for grazing livestock, as well as for those who use such plants for medicinal purposes. Feeding experiments with plant material, crude total alkaloids, and certain pyrrolizidine alkaloids of known structure have shown that alkaloids of this type are often responsible for marked toxicities and death in man and animals. Several authors have reviewed the toxicity of pyrrolizidine alkaloids through 1970 (186–188). Until that time, there was a lack of agreement as to the oncogenic nature of pyrrolizidine alkaloids (187). However, evidence in support of the oncogenicity of *certain types* of these alkaloids now appears to be at hand (table 5).

It had been postulated that pyrrolizidine alkaloids were not oncogenic, since in acute pyrrolizidine alkaloid poisoning in sheep, cows, horses, pigs, and fowl (187), no liver or other types of tumors were found. However, at that time there was some disagreement over the morphologic definition of hepatomas produced by certain pyrrolizidine alkaloids (187,189). More recent studies have shown that certain pyrrolizidine alkaloids are associated with induction of renal (190), central nervous system (191), pancreatic (192), and epithelial tumors (193), as well as rhabdomyosarcomas (194).

An inspection of the structures of those alkaloids that were shown to be oncogenic (figs 2–6) does not allow one to draw any conclusion concerning oncogenic structure-activity relationships. In addition, the non-naturally occurring dehydroretronecine, with unsaturation at the 1,8- and 2,3- positions, is oncogenic (194). Thus, the pyrrolizidine nucleus apparently does not even require specific sites of unsaturation. It is interesting to note that two of the nine oncogenic pyrrolizidine alkaloids, ie, lasiocarpine (XXIII) (195) and monocrotaline (XVII) (195–197), also have antitumor activity.

Sanguinarine as an Oncogenic Agent

Hakim (206) has extensively reviewed the toxicity of *Argemone mexicana* L. seed oil, used either by itself or mixed with other fixed oils for cooking. His recommendation to ban the use of *Argemone* oil is supported by an exhaustive bibliography showing that the culinary use of this oil is associated with deaths, cardiomyopathies, glaucoma, and possibly cancer. In 1948, both sanguinarine (XXIV) and dihydrosanguinarine were isolated from the oil of *Argemone mexicana* seeds in quantities of about 9.5 mg/ml (207), and sanguinarine has been reported to be oncogenic in rats, guinea pigs, and hamsters (206). Sarcomas have been induced in rats with a single 0.05-ml injection of *Argemone* oil (206).

Analysis of urine from sanguinarine-treated rabbits revealed a green fluorescent metabolite, which Hakim et al (208) tentatively identified as 3,4-benzacridine (scheme 3). Although there is some question as to the identity of this sanguinarine metabolite (209), it is clear that sanguinarine must be considered an oncogenic substance.

It is interesting to note that sanguinarine and related benzophenanthridine alkaloids have also shown anticancer activity in laboratory animals (*see* fig 7) (183,210,211).

Nitrosamines as Oncogenic Agents

The Bantu people in certain areas of the Transkei have experienced an increased incidence of esophageal cancer since the early 1940s. An association was noted with respect to the incidence of esophageal cancer in women and signs of molybdenum deficiency in the leaves of certain food plants such as pumpkins, beans, and maize. Since molybdenum is essential for nitrogen metabolism in plants, an accumulation of nitrates would be expected in its absence. Reaction of the nitrate with secondary amines in the plants would thus be expected to result in the formation of oncogenic nitrosamines (212).

This hypothesis led Du Plessis and co-workers to examine the food and medicinal plants in or near the villages of the Bantu people for the presence of nitrosamines. It was found that the fruit juice of *Solanum incanum* L. was used to curdle milk, the resulting curd being a major protein source in the diet of the Bantu. Dimethylnitrosamine (XXIX) was identified in several samples of *S. incanum* fruit and may account for the high incidence of esophageal cancer in many of the Bantu people (212).

Table 5. — Summary of tumors in animals following pyrrolizidine alkaloid administration

Material	Dose	Route	Species	Type of tumor	Ref.
Amsinckia intermedia Fisch. & Meyer					
(Total alkaloids)	500-1500 mg/kg	Oral	Rat	Pancreatic islet	192
(Seeds)	In diet	Oral	Rat	Lipomatous renal tumor	198
Heliotropium crispum Desf. [*H. ramosissimum* (Lehm.) Sieber ex A.DC.]					
(Whole plant)	5.0% of diet	Oral	Rat*	Spinal cord tumor	191
Heliotropium supinum L.					
(Leaves & stems)	10% of diet	Oral	Rat	Lipomatous renal tumor	192
(Alkaloid fraction)	400 mg/kg (once)	Oral	Rat	Lipomatous renal tumor	190
Senecio aquaticus Hill	1.0% of diet	Oral	Rat	Lipomatous renal tumor	189
Senecio douglasii DC. var. *longilobus* (Benth.) L.Benson [*S. longilobus* Benth.]	0.25 to 5% of diet	Oral	Rat	Hepatomas	190
Senecio jacobaea L.					
(Crude alkaloids)	0.05-0.1 mg/ml	Oral	Rat	Hepatomas	200
(Crude alkaloids)	0.05 mg/ml, 3 days/week	Oral	Rat	Hepatomas	199
(Crude alkaloids)	0.5 LD50/week for 8 weeks	iv	Fowl	Hepatomas	201
(Milled plant)	3.5-7.5% of diet	Oral	Fowl	Hepatomas	201
Retronecine (XV)	0.02-0.05 ml (50 mg/ml)/newborn	sc	Rat	Spinal cord tumor	191
Dehydroretronecine (XVI)	20 mg/kg twice weekly for 4 months, then 10 mg/kg for 8 months	sc	Rat	Rhabdomyosarcomas	194
Monocrotaline (XVII)	0.03 mg/ml in drinking water	Oral	Rat	Hepatomas	202
	4-12 mg/kg/1 yr.	ip	Rat	Hepatomas	202
	5 mg/kg twice weekly/12 months	sc	Rat	Rhabdomyosarcomas	194
Retrorsine (XVIII)	30 mg/kg (once) + X-ray	Oral	Rat	Hepatomas;metastatic liver tumors	203
	3 doses within 10 months (30, 30 & 8 mg/kg)	Oral	Rat	Lipomatous liver	190
	25-30 mg/kg, 6 doses over 5 months	ip	Rat	Hepatomas	204
	0.03 mg/ml, 3 times/week	Oral	Rat	Hepatomas	199
Isatidine (XIX)	0.03 mg/ml in drinking water	Oral	Rat	Hepatomas; metastatic liver tumors	199, 204
	25-30 mg/kg, 5 doses over 2 months	ip	Rat	Hepatomas	203
	5% (w/v) solution to skin 3 X weekly/ 15 months	Topical	Rat	Hepatomas	199
Hydroxysenkirkine (XX)	100-300 mg/kg (once)	sc	Rat	Astrocytomas	191
Jacobine (XXI)	-	Oral	Fowl	Hepatomas	201
Seneciphylline (XXII)	-	Oral	Fowl	Hepatomas	201
Lasiocarpine (XXIII)	7.8 mg/kg twice weekly/4 weeks, then once/week/52 weeks	ip	Rat	Hepatomas;squamous cell carcinomas	193,205

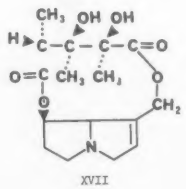
*From litter of treated rat

XV-XVI

XV Retronecine $\Delta^{1,2}$

XVI Dehydroretronecine $\Delta^{1,8}$, $\Delta^{2,3}$

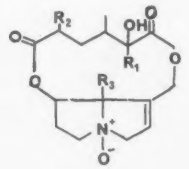
FIGURE 2.—Oncogenic non-ester pyrrolizidine alkaloids.



XVII

XVII Monocrotaline

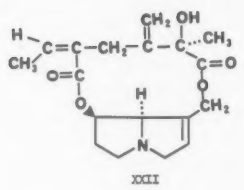
FIGURE 3.—Cyclic diester pyrrolizidine alkaloid.



XVIII-XXI

		R	R_1	R_2	R_3
XVIII	Retrorsine	-	$-CH_2OH$	$=CH-CH_3$	...H
XIX	Isatidine	=O	$-CH_2OH$	$=CH-CH_3$	...H
XX	Hydroxysenkirkine	CH_3	$...CH_2OH$	$=CH-CH_3$	...O
XXI	Jacobine	-	$-CH_3$	$-CH-CH_3$	...H
				-O	

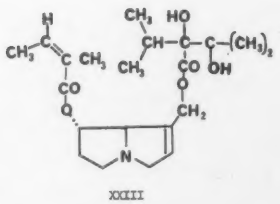
FIGURE 4.—Oncogenic cyclic diester pyrrolizidine alkaloids.



XXII

XXII Senecitphylline

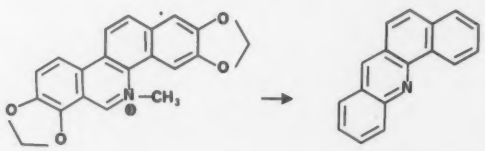
FIGURE 5.—Oncogenic acyclic diester pyrrolizidine alkaloid.



XXIII

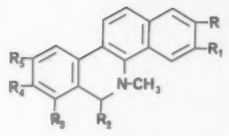
XXIII Lasiocarpine

FIGURE 6.—Oncogenic acyclic diester pyrrolizidine alkaloid.



XXIV

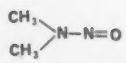
SCHEME 3.—Suggested metabolic fate of sanguinarine (207).



XXV-XXVIII

Benzophenanthridine Alkaloid	Substituents						
	R	R_1	R_2	R_3	R_4	R_5	Other
Fagaronine (XXV)	OH	OCH_3	H	H	OCH_3	OCH_3	$\Delta^{7,8}$, N^+
Methylfagaronine (XXVI)	OCH_3	OCH_3	H	H	OCH_3	OCH_3	$\Delta^{7,8}$, N^+
Nitidine (XXVII)	$-O-CH_2-O-$		H	H	OCH_3	OCH_3	$\Delta^{7,8}$, N^+
8-Methoxydihydronitidine (XXVIII)	$-O-CH_2-O-$		OCH_3	H	OCH_3	OCH_3	
Sanguinarine (XXIV)	$-O-CH_2-O-$		H	$-O-CH_2-O-$		H	$\Delta^{7,8}$, N^+

FIGURE 7.—Structures of antitumor benzophenanthridine alkaloids.



XXIX

Safrole as an Oncogenic Agent

Safrole (4-allyl-1,2-methylenedioxybenzene; XXX) is widely distributed in plants, constituting some 70%–80% of the flavoring agent, sassafras oil. Oil of sassafras and safrole were widely used in the US to flavor soft drinks, primarily root beer, until 1960. In that year, the use of safrole was banned (213) by the US Food and Drug Administration, on the grounds that several investigators had reported it to be a weak oncogen in the rat and mouse (213–226). From 0.04% to 1.0% of safrole in the male or female rat diet will produce hepatic adenomas and hepatic carcinomas (223). A variety of metabolic products of safrole (fig 8) have been identified in the rat (227–233), mouse (229,234–236), and guinea pig (228,232,233).

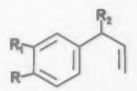
Of these metabolites, 1′-hydroxysafrole (XXVIII) (229,230,234,236) and dihydrosafrole (XXXV) are oncogenic (221,237).

Rotenone as an Oncogenic Agent

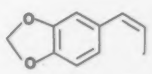
It has been reported that rotenone (XLI) injected into female rats (1.7 μg/g/day for 42 days ip) induces the formation of mammary tumors in 60%–100% of rats so treated (238).

Asiaticoside as an Oncogenic Agent

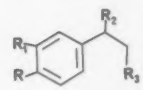
Asiaticoside (XLII), a triterpene glycoside isolated from *Centella asiatica* (L.)Urban, has been reported



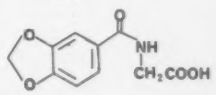
A



XXXIV



B



XXXVIII

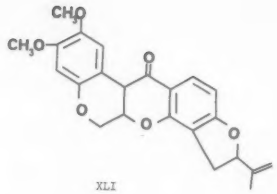
A Structures

	R	R_1	R_2
XXX	$-CH_2-O-CH_2-$		-H
XXXI	-OH	-OH	-H
XXXIII	$-CH_2-O-CH_2-$		-OH

B Structures

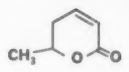
	R	R_1	R_2	R_3
XXXV	$-CH_2-O-CH_2-$		-H	$-CH_3$
XXXVI	$-CH_2-O-CH_2-$		-OH	$-CH_2OH$
XXXVII	$-CH_2-O-CH_2-$		-OH	-COOH
XXXIX	$-CH_2-O-CH_2-$		=O	$-CH_2-N-(CH_3)_2$
XL	$-CH_2-O-CH_2-$		=O	$-CH_2-N$

FIGURE 8.—Major metabolites of safrole: XXX, safrole; XXXI, 1,2-dihydroxy-4-allylbenzene; XXXII, 1,2-dihydroxy-4-(2,3-dihydroxypropyl)benzene; XXXIII, 1′-hydroxysafrole; XXXIX, isosafrole; XXXV, dihydrosafrole; XXXVI, 1,2-methylenedioxy-4-(2,3-dihydroxypropyl)benzene; XXXVII, 2-hydroxy-3-(3′,4′-methylenedioxypheriyl)propionic acid; XXXVIII, 3,4-methylenedioxybenzoylglycine; XXXIX, 3-*N*,*N*-dimethylamino-1-(3′,4′-methylenedioxyphenyl)-1-propanone; XL, 3-piperidyl-1-(3′,4′-methylenedioxyphenyl)-1-propanone.



XLI

to have a low toxicity; a few applications to guinea pig skin showed cicatrization and a growth-stimulant action but no other unusual effects (239,240). Because of the stimulant effect of asiaticoside, Laerum and Iversen (241) studied the compound in a new type of short-term oncogen test. They found that its application to the skin of mice as a 0.1% solution in benzene increased the yield of papillomas and induced 2.5% of skin sarcomas.



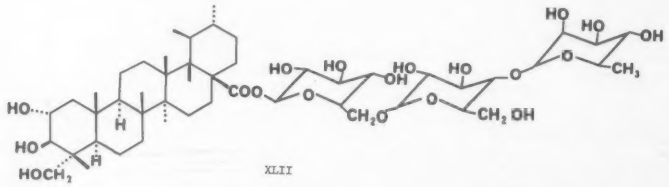
XLIV

Phytohemagglutinin as a Tumor-Enhancing Agent

Phytohemagglutinin (unspecified source) given in large doses to mice bearing 20-methylcholanthrene-induced fibrosarcomas was found to significantly enhance tumor growth (244).

Miscellaneous Plant Products as Oncogens

In addition to the compounds discussed above,



XLII

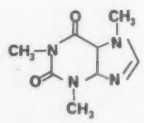
numerous other plant principles have been shown to be oncogenic in animals including certain antitumor agents such as podophyllotoxin, vinblastine, colchicine, and demecolcine (245–248). This is not unexpected, since almost all clinically useful antitumor agents, both natural and synthetic, are also carcinogenic (249). Moreover, under specialized test conditions a large number of common natural products are oncogens, including many common carbohydrates, amino acids, lipids, sterols, flavonoids, and alkaloids such as reserpine, quinine, ephedrine, and others (245–249).

The major oncogenic products from plants are summarized in table 6, and plants shown to be oncogenic on the basis of animal testing are presented in table 7.

A survey of the literature was conducted to determine the distribution in plants of the major oncogenic substances reported in table 6, and a summary of the findings is shown in table 8. Since known oncogenic substances are present in these plants, each must be considered as potentially oncogenic even though direct experimental data are lacking.

Caffeine as a Potential Oncogen

It has been reported recently that caffeine (XLIII) enhances the frequency of oncogenic human adenovirus type-12 transformation of hamster embryo cells (242), as well as the transformation of hamster cells by several chemical carcinogens (243). The significance of these effects remains to be determined.



XLIII

Parasorbic Acid as an Oncogen

Parasorbic acid (XLIV), initially isolated from the fruits of *Sorbus aucuparia* L., is reported to have induced sarcomas in 11 of 12 rats following sc injection (12).

Table 6. —Summary of oncogenic compounds isolated from angiosperms, gymnosperms, and pteridophytes

Compounds	Structure	Ref.
Alkaloids		
Colchicine	-	244-249
Demecolcine	-	244-249
Hydroxysenkirkine	XX	191
Isatidine	XIX	199, 204
Jacobine	XXI	201
Lasiocarpine	XXIII	193, 205
Monocrotaline	XVII	194, 202
Quinine	-	244-247
Reserpine	-	244-247
Retronecine	XV	191
Retrorsine	XVIII	190, 199, 203, 204
Sanguinarine	XXIV	206
Seneciphylline	XXII	201
Vincaleukoblastine	-	248
Nitroso derivatives		
Cycasin	IV	64, 65
Dimethylnitrosamine	XXIX	212
Macrozamin	III	63, 68
Methylazoxymethanol	IX	72
Neocycasin A	V	67
Neocycasin B	VI	68
Neocycasin C	VII	69
Neocycasin E	VIII	70-71
N'-Nitrosonornicotine	I	13
Triterpenes		
Asiaticoside	XLII	240
Miscellaneous		
Parasorbic acid	XLIV	12
Podophyllotoxin	-	244-248
Rotenone	XLI	238
Safrole	XXX	213-226
Shikimic acid	II	59

Table 7. - Plants eliciting oncogenic activity in animals

Family	Plant	Part*	Carcinogenic constituent	Route of administration**	Ref.
		PTERIDOPHYTA			
Polypodiaceae	*Pteridium aquilinum* (L.) Kuhn	FD	Shikimic acid	in ration	51,58 59
		RZ		in ration	58
		SK		in ration	58
		GYMNOSPERMAE			
Cycadaceae	*Cycas circinalis* L.	SD	cycasin	in ration	73
		HK	cycasin	in ration	76,77
		FR	cycasin	in ration	93
		SM	cycasin	in ration	74,75
	Cycas revoluta Thunb.	-	cycasin	-	12
		ANGIOSPERMAE			
Anacardiaceae	*Schinopsis quebracho-colorado* (Schlecht.) Barkley & Mayer [*S. lorentzii* (Griseb.)Engl.]	LF	tannins	sc	167
Annonaceae	*Annona muricata* L.	LF		hb,im	170,172
Boraginaceae	*Amsinckia intermedia* Fisch. & Mayer	SD		in ration	198
		PA		oral	192
	Heliotropium angiospermum	PX		sc	171
	Heliotropium crispum Desf. J.A.Murray [*H. ramosissimum* (Lehm.) Sieber ex A.DC.]	PL		oral in ration	191
	Heliotropium supinum L.	LF,ST		in ration	192
		PA		oral	190
	Heliotropium ternatum Vahl	LF		hb,im	170,172
Chenopodiaceae	*Beta vulgaris* L.	RT		-	250
Combretaceae	*Terminalia chebula* Retz.	LF	tannins	sc	167
Compositae	*Petasites japonicus* (Sieb. & Zucc.) Maxim.	FS		in ration	251
	Senecio aquaticus Hill	PL		in ration	189
	Senecio douglasii DC. var. *longilobus* (Benth.) L.Benson [*S. longilobus* Benth.]	ST,LF		in ration	190
Fagaceae	*Castanea sativa* Mill.	LF	tannins	sc	167
	Quercus aegilops L.	LF	tannins	sc	167
Krameriaceae	*Krameria ixina* L.	PX	tannins	hb,im,sc	170-173
	Krameria triandra Ruiz & Pavon	RT	tannins	sc	173
Leguminosae	*Acacia pubescens* (Vent.)R.BR. [*A. molissima* Willd.]	LF	tannins	sc	167
	Acacia villosa (Sw.) Willd.	LF	tannins	hb,sc	171-173
	Gliricidia sepium (Jacq.) Kunth ex. Steud.	LF		im	170
Loranthaceae	*Viscum album* L.	ST		-	250
Myrtaceae	*Eucalyptus reduncа* Schauer	LF	tannins	sc	167
Papaveraceae	*Argemone mexicana* L.	SO	sanguinarine	im	206,207
Rutaceae	*Citrus aurantium* L.	LF		im	170
Sterculiaceae	*Melochia tomentosa*	RT		sc	171
Tannic acid	-	-	-	sc	156-167

*FD = frond, FR = fruit, FS = fruit shell, HK = husk, LF = leaves, PA = mixture of pyrrolizidine alkaloids, PL = whole plant, PX = aerial parts, RT = root, RZ = rhizome, SD = seed, SK = stalk, SM = seed meal, SO = seed oil, ST = stem.

**sc = subcutaneous, hb = hamster buccal pouch, im = intramuscular.

Table 8. — The Distribution of Selected Major Oncogens in the Plant Kingdom

Plant name	Part[b]	Oncogen	Ref.
	PTERIDOPHYTA		
POLYPODIACEAE			
Pteridium aquilinum (L.) Kuhn [*Pteris aquilina* L.]	Fd,Rz	shikimic acid	59
	GYMNOSPERMAE		
CUPRESSACEAE			
Callitris drummondii (Parl.) F.Muell.	-	podophyllotoxin	252
Juniperus lucayana Britt[a]	LF	podophyllotoxin	255
Juniperus pseudosabina Fisch. & Meyer	-	podophyllotoxin	253
Juniperus sabina L.	LF	podophyllotoxin	253,254
Juniperus sabina var. *tamariscifolia* Ait.	LF	podophyllotoxin	255
Juniperus scopulorum Sarg.	LF	podophyllotoxin	255
Juniperus silicicola (Small) Bailey	LF	podophyllotoxin	255
Juniperus virginiana L.	LF	podophyllotoxin	256
CYCADACEAE			
Bowenia serrulata (Bull) Chamberl. [*B. serrata* F.M.Bailey]	Sd	macrozamin	257
Bowenia spectabilis Hook.	Sd	macrozamin	257
Cycas circinalis L.	Sd	cycasin	66
	Nt	cycasin	73,258
	-	methylazoxymethanol	72
	En	cycasin	259
	Sn	cycasin	260
	Kn	cycasin	261
Cycas media R.Br.	Sd	macrozamin	257
Cycas revoluta Thunb.	Us	cycasin	262
	Hs	cycasin	64,263
	Sd,St	cycasin	65
	Sl	macrozamin	68,264
	-	neocycasin B	68,264
	Sd	neocycasin A	67,264
	-	neocycasin B	67,264
	-	neocycasin C	69
	-	neocycasin E	71
Encephalartos hildebrandtii A.BR. & Bouche	Sd	macrozamin	265
Encephalartos lanatus Stapf & Burtt-Davy	Sd	macrozamin	266
Encephalartos transvenosus Stapf & Burtt-Davy	Sd	macrozamin	266

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Macrozamia douglasii T.Hill ex F.M.Bailey	Sd	macrozamin	257
Macrozamia hopei T.Hill ex F.M.Bailey	Sd	macrozamin	257
Macrozamia miquelii (F.Muell.) A.DC.	Sd	macrozamin	257
Macrozamia moorei F.Muell. ex C.Moore	Sd	macrozamin	257
Macrozamia pauli-guilielmi W.Hill & F.Muell.	Sd	macrozamin	257
Macrozamia riedlei (Fisch. ex Gaud.-Beaup.) C.A.Gardn.	Sd	macrozamin	267
Macrozamia spiralis (Salisb.) Miq.	-	macrozamin	63
ANGIOSPERMAE - MONOCOTYLEDONEAE			
LILIACEAE			
Androcymbium graminaum (Cav.) Macbride	Co,Fl,Lf,Pl,Sd	colchicine	268-270
Androcymbium melanthioides Willd.	Co	colchicine	271
Androcymbium melanthioides var. *stricta*[a]	Sd	colchicine	269
Asphodelus sp.	-	colchicine	272
Bulbocodium ruthenicum Bunge ["*Colchicum ruthenicum*"]	Lf	colchicine	273
Bulbocodium vernum L. [*Colchicum vernum* Ker]	Co,Fl,Lf	colchicine	269,270,273
Camptorrhiza strumosa (Baker) Oberm.	Co,Pl,Sd	colchicine	269,271,274
Colchicum agrippinum Hort. ex Baker	Bu,Co	colchicine	269,270,273,275
Colchicum alpinum Lam. & DC. [*C. montanum* All.]	Bu,Co	colchicine	269,271,273
Colchicum arenarium Waldst. & Kit.	Bu,Co,Fl,Pl,Sd	colchicine	269,270,273
	Bu,Co,Fl,Pl,Sd,St	demecolcine	269,270,276
Colchicum autumnale L.	Bu,Co,Fl,Lf,Pc,Sd	colchicine	269,270,277-279
	Bu,Co,Fl,Pc,Pl,St	demecolcine	269,270,280
Colchicum autumnale var. *album* Hort.	Co	colchicine	269
	Co	demecolcine	269,270
Colchicum autumnale var. *flore-pleno* L.	Co	colchicine	269
	Co	demecolcine	269,270
Colchicum autumnale var. *major* Hort.	Co	colchicine	269
	Co	demecolcine	269,270
Colchicum autumnale var. *minor* Hort.	Fl	colchicine	269
Colchicum bivonae Guss.	Sd	colchicine	273
Colchicum byzantium Ker	Co,Fl	colchicine	281
	Co,Fl	demecolcine	271
Colchicum cornigerum (Schweinf.) Tackh. & Drar	Co,Sd	colchicine	269,282
	Fl,Lf,Sd	demecolcine	271,283
Colchicum crociflorum H.W.Schott & T.Kotschy	Co	colchicine	269,270,284
Colchicum cupanii Guss.	Pl	colchicine	273,281
Colchicum doerfleri Halacsy ex Degen. & Doerfl.	Sd	colchicine	285,286
Colchicum hierosolymitanum Feinbr.	Bu,Co	colchicine	269,270,273
	Co,Sd	demecolcine	269,270

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part [b]	Oncogen	Ref.
Colchicum kesselringii Regel ["*Merendera kesselringii*"]	Co,Lf,Sd	colchicine	270,273,287-289
Colchicum kotschyi Boiss. [*C. persicum* Baker]	Sd	colchicine	273
Colchicum lastum Stev.	Co,Sd	colchicine	281
Colchicum libanoticum Ehren. ex Boiss.	-	colchicine	281
Colchicum lusitanicum Brot.	Bu,Co,Fl	colchicine	269,270
Colchicum luteum Baker	Co,Pl,Px,Sd	colchicine	269-271,290-292
Colchicum macedonicum Kosanin	Sd,Tu	colchicine	269,271,273,294
Colchicum neapolitanum Tenore	Bu,Sd	colchicine	273
Colchicum ritchii R.Br.	Co,Pl	colchicine	269,271
Colchicum speciosum Stev. [*C. borrmuelleri* Freyn.]	Bu,Co,Fl,Lf	colchicine	269,270,273,295
	Bu,Co,Fl	demecolcine	269,295,296
Colchicum speciosum Stev. [*C. veratrifolium* S.Arn.]	Sd	colchicine	273
Colchicum stevenii Kunth [*C. cilicum* Hayek & Siehe]	Bu,Co	colchicine	269,270,273
Colchicum tenorii Parl. [*C. bisignanii* Tenore]	Sd	colchicine	273
Colchicum tunicatum Feinbr.	Pl	colchicine	269
	Pl	demecolcine	269
Colchicum variegatum L.	Bu,Co,Sd	colchicine	269,270,273
Colchicum 'Disraeli'	Co,Fl	colchicine	269
	Co	demecolcine	269
Colchicum 'Lilac wonder'	Co	colchicine	269
	Co	demecolcine	270
Colchicum 'The Giant'	Co	colchicine	269
	Co	demecolcine	270
Colchicum 'Violet Green'	Co	colchicine	269
	Co	demecolcine	270
Dipidax triquetra (L.fil.) Baker	Co,Sd	colchicine	269,270
Fritillaria sp.	-	colchicine	272
Gloriosa simplex L.	Co,Tu	colchicine	269,270,297
Gloriosa superba L.	Co,Tu		269,270,297,298
Gloriosa virescens Lindl.	Co,Sd	colchicine	271
Hemerocallis sp.	-	colchicine	273
Iphigenia bechuanica Baker	-	colchicine	274
Iphigenia indica (L.) Gray ex Kunth	Co,Pl	demecolcine	269,271,274
	-	colchicine	269,271,274
Iphigenia pallida Baker	Co,Pl	demecolcine	269,271,274
	-	colchicine	269,271,274
Kreysigia multiflora (R.Br.) Reichb.	Lf,Pl,Rt,St	colchicine	299

Table 6.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref
Littonia modesta Hook.fil.	Co,Lf,Sd,St	colchicine	269-271
Merendera attica (Sprun. ex Tommas.) Boiss & Sprun.	Bu,Co	colchicine	269,270,275, 284
Merendera caucasica Bieb.	Bu,Co,Lf	colchicine	270,273
Merendera montana (L.) Lange [*M. bulbocodium* Ramond]	Bu,Co,Fl	colchicine	269,271,273
Merendera persica Boiss. & Kotschy	Co	demecolcine	271
Merendera raddeana Regel	-	colchicine	300
Merendera robusta Bunge	Bu,Lf,Pl,Sd	colchicine	271,273
	Pl	demecolcine	269,271
Merendera sobolifera Fisch. & Meyer [*Colchicum sobolifernm* (C.A.Meyer) Staf.]	Bu,Co,Lf,Sd	colchicine	269,273,285
	Co	demecolcine	269
Merendera trigyna (Adams) Woronow	Co,Sd	colchicine	270,284
Muscari sp.	-	colchicine	272,301
Ornithogloseum sp.	-	colchicine	272
Ornithogloseum viride (L.fil.) Dryand [*O. glaucum* Salisb. var. *grandiflorum* Baker]	Co	colchicine	269,271
Tofieldia sp.	-	colchicine	272
Tulipa sp.	-	colchicine	301
ANGIOSPERMAE - DICOTYLEDONEAE			
ANACARDIACEAE			
Schinopsis quebracho-colorado (Schlecht.) Barkley & Meyer [*S. lorentzii* (Griseb.) Engl.]	Lf	tannins	167
Schinus weinmanniifolius Mart. ex Engl.	Vo	safrole	302
APOCYNACEAE			
Alstonia constricta F.Muell.	Rb	reserpine	303,304
Alstonia venenata R.Br.	Bk,Sb	reserpine	305-307
Aspidosperma auriculatum Markgraf	-	reserpine	308
Blaeksria coccinea (Teysm. & Binnend.) Koidz. [*Excavatia coccinea* (Teysm. & Binnend.) Markgraf]	-	reserpine	310
Catharanthus ovalis Markgraf	Pl	vincaleukoblastine	309
Catharanthus roseus (L.) G.Don [*Vinca rosea* L.]	Rt	reserpine	309
	Lf	vincaleukoblastine	309
Ochrosia poweri F.M.Bailey	Bk,Lf	reserpine	311
Rauvolfia amoniifolia A.DC.	Rt	reserpine	273,313
Rauvolfia bahiensis A.DC.	Rt	reserpine	312
Rauvolfia caffra Sonder [*R. inebrians* K.Schum.]	Rt	reserpine	273,312,315
Rauvolfia caffra Sonder [*R. natalensis* Sonder]	Rb	reserpine	323

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Rauvolfia cambodiana Pierre ex Pitard	Rz	reserpine	316,317
Rauvolfia cubana A.DC.	Rt	reserpine	312
Rauvolfia cumminsii Stapf	Rb	reserpine	273
Rauvolfia decurva Hook.fil.	Rt	reserpine	273
Rauvolfia densiflora (Wall.) Benth. ex Hook.fil.	Rt	reserpine	273
Rauvolfia grandiflora Mart. ex A.DC. [*R. affinis* Muell.-Arg.]	-	reserpine	312
Rauvolfia ligustrina Willd. ex Roem. & Schult. [*R. indecora* Woods.]	Rt	reserpine	312
Rauvolfia ligustrina Willd. ex Roem. & Schult. [*R. ternifolia* H.B.K.]	Rt	reserpine	332
Rauvolfia littoralis Rusby	Rt	reserpine	312
Rauvolfia macrophylla Stapf	Rt	reserpine	273
Rauvolfia mannii Stapf	Rt	reserpine	273
Rauvolfia micrantha Hook.fil.	-	reserpine	321
Rauvolfia mombasiana Stapf	Rt,Ws	reserpine	322
Rauvolfia nana E.A.Bruce	Rt	reserpine	273
Rauvolfia nitida Jacq.	Rt	reserpine	312
Rauvolfia obscura K.Schum.	Rt,Rb	reserpine	312,324
Rauvolfia oreogiton Markgraf	Rb	reserpine	325
Rauvolfia paraensis Ducke	Rt	reserpine	312
Rauvolfia pentaphylla Ducke	Rb	reserpine	312
Rauvolfia perakensis King & Gamble	Rt	reserpine	326,327
Rauvolfia rosea K.Schum.	Rt	reserpine	312
Rauvolfia salicifolia Griseb.	Rt	reserpine	312
Rauvolfia sandwicensis A.DC.	Rt	reserpine	312
Rauvolfia sarapiquensis Woods.	-	reserpine	273
Rauvolfia schuelii Spegaz [*R. boliviana* Markgraf]	Rb	reserpine	273,314
Rauvolfia sellowii Muell.-Arg.	Rt,Rb	reserpine	328-330
Rauvolfia serpentina (L.) Benth. ex Kurz	Rt	reserpine	331
Rauvolfia sprucei Muell.-Arg.	Rt	reserpine	312
Rauvolfia sumatrana Jack	Rt	reserpine	332
Rauvolfia tetraphylla L. [*R. canescens* L.]	Rt	reserpine	318,319
Rauvolfia tetraphylla L. [*R. hirsuta* Jacq.]	Rt	reserpine	273
Rauvolfia verticillata (Lour.) Baill. [*R. chinensis* (Hance) Hemsl.]	Rt	reserpine	320
Rauvolfia viridis Willd. ex Roem. & Schult. *R. lamarckii* A.DC.	Rt	reserpine	312
Rauvolfia vomitoria Afzel.	Rt,Rb	reserpine	337-339
Rauvolfia yunnanensis Tsiang	Rt	reserpine	340
Tonduzia longifolia (A.DC.) Woods	-	reserpine	308

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Vallesia glabra (Cav.) Link [*V. dichotoma* Ruiz & Pav.]	-	reserpine	341
Vinca herbacea Waldst. & Kit.	Pl	reserpine	342
Vinca major L.	Rt	reserpine	343
Vinca minor L.	Lf,Rt	reserpine	344-345
Voacanga africana Stapf ex Elliot	-	reserpine	346
ARISTOLOCHIACEAE			
Asarum sp.[a] [*Heterotropa controversa*]	Vo	safrole	352
Asarum albivenium Regel [*Heterotropa albivenium* (Regel)]	Vo	safrole	350
Asarum asaroides (Morr. & Decne.) Makino [*Heterotropa asaroides* Morr. & Decne.]	Vo	safrole	351
Asarum asperum Maekawa [*Heterotropa aspera* (Maekawa) Maekawa]	Vo	safrole	352
Asarum caulescens Maxim.	Vo	safrole	347
Asarum constrictum Maekawa [*Heterotropa constricta* (Maekawa) Maekawa]	Vo	safrole	352
Asarum crassum Maekawa [*Heterotropa crassa* (Maekawa) Maekawa]	Vo	safrole	352
Asarum curvistigma Maekawa [*Heterotropa curvistigma* (Maekawa) Maekawa]	Vo	safrole	351
Asarum dimidiatum Maekawa [*Asiasarum dimidiatum* (Maekawa) Maekawa]	Vo	safrole	347
Asarum fauriei Franch. [*Heterotropa fauriei* (Franch.) Maekawa]	Vo	safrole	350
Asarum grandiflorum Hayata [*Heterotropa hayatana* Maekawa]	Vo	safrole	352
Asarum hexalobum Maekawa [*Heterotropa hexaloba* (Maekawa) Maekawa]	Vo	safrole	352
Asarum hexalobum var. *perfectum* Maekawa [*Heterotropa hexaloba* Maekawa var. *perfecta* (Maekawa)]	Vo	safrole	352
Asarum kiusianum Maekawa [*Heterotropa kiusiana* (Maekawa) Maekawa]	Vo	safrole	351
Asarum kurosawae suginoto[a] [*Heterotropa kurosawae*]	Vo	safrole	350
Asarum leptophyllum Hayata	-	safrole	347
Asarum muramatsui Makino [*Heterotropa muramatsui* (Makino) Maekawa]	Vo	safrole	351
Asarum nipponicum Maekawa [*Heterotropa nipponica* (Maekawa) Maekawa]	Vo	safrole	350
Asarum nipponicum var. *brachypodion* Maekawa [*Heterotropa nipponica* var. *brachypodion*]	Vo	safrole	350
Asarum nipponicum var. *kooyanum* (Makino) Maekawa [*Heterotropa nipponica* var. *kooyana* (Makino) Maekawa]	Vo	safrole	350
Asarum oblongum (Maekawa) O.C.Schmidt [*Heterotropa oblonga* Maekawa]	-	safrole	352
Asarum rigescens Maekawa [*Heterotropa rigescens* (Maekawa) Maekawa ex Nemoto]	Vo	safrole	352

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Asarum sakawanum Makino [*Heterotropa sakawana* (Makino) Maekawa]	Vo	safrole	352
Asarum satsumense Maekawa [*Heterotropa satsumensis* (Maekawa) Maekawa]	Vo	safrole	351
Asarum savatieri Franch. [*Heterotropa savatieri* (Franch.) Maekawa]	Vo	safrole	350
Asarum savatieri [*Heterotropa savatieri* var. *furusei*]	Vo	safrole	350
Asarum sieboldii Miq.	Vo	safrole	348
Asarum sieboldii Miq. var. *cineoliferum* Fujita [*Asiasarum heterotropoides* var. *cineoliferum*]	Vo	safrole	347
Asarum sieboldii var. *mandshuricum* (Maxim.)Kitagawa [*Asiasarum heterotropoides* var. *mandshuricum*]	Vo	safrole	347
Asarum sieboldii var. *seoulensis* [*Asiasarum heterotropoides* var. *seoulense* (Nakai) Maekawa]	Vo	safrole	347,349
Asarum subglobosum Maekawa [*Heterotropa subglobosa* Maekawa]	Vo	safrole	351
Asarum tamaense Makino [*Heterotropa tamaensis* (Makino) Maekawa]	Vo	safrole	351
Asarum unzen (Maekawa) Kitamura & Murata [*Hetrotropa unzen* Maekawa]	Vo	safrole	351
Asarum variegatum A.Br. & Bouche [*Heterotropa variegata* (A.Br. & Bouche) Maekawa ex Nemoto]	Vo	safrole	350
Asarum yakusimense Masamune [*Heterotropa yakusimensis* (Masamune) Maekawa ex Nemoto]	Vo	safrole	351
BERBERIDACEAE			
Diphylleia grayi F.Schmidt	Rt	podophyllotoxin	361
Podophyllum emodi var. *hexandrum*[a]	Rt	podophyllotoxin	366
Podophyllum hexandrum Royle [*P. emodi* Wall. ex Royle]	Rt	podophyllotoxin	366
Podophyllum indicum[a]	Rt	podophyllotoxin	367
Podophyllum peltatum L.	Rt,Rz	podophyllotoxin	368-373
Podophyllum pleianthum Hance	Rt,Rz	podophyllotoxin	374
BORAGINACEAE			
Cynoglossum officinale L.	Pl	lasiocarpine	354
Heliotropium arborescens L.	-	lasiocarpine	355
Heliotropium europaeum L.	Pl,Sd	lasiocarpine	356,357
Heliotropium indicum L.	Pl	retronecine	353
Heliotropium lasiocarpum Fisch. & Meyer	-	lasiocarpine	358
Lappula intermedia (Ledeb.) Popov	Lf,St	lasiocarpine	359
Symphytum caucasicum Bieb.	-	lasiocarpine	360
BURSERACEAE			
Protium brasiliense (Spreng.) Engl.	Sd	safrole	375
COMBRETACEAE			
Terminalia chebula Retz.	Lf	tannins	167

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
COMPOSITAE			
Adenostyles rhombifolia (Willd.) Pimenov [*Senecio platyphyllus* DC.]	Pl,Rt,Rz	seneciphylline	418-421
Adenostyles rhombifolia [*Senecio rhombifolius* (Willd.) Sch.-Bip.]	Px,Rt,Rz	seneciphylline	414
Erechtites hieracifolia (L.) Rafin. ex DC.	-	seneciphylline	376
Ligularia stenocephala (Maxim.) Matsum. & Koidz [*Senecio stenocephalus* Maxim.]	-	seneciphylline	400
Senecio adonidifolius Loisel.	Px	seneciphylline	379
Senecio alpinus (L.) Scop.	-	seneciphylline	380
Senecio ambraceus Turcz. ex DC. [*S. krylovii* Schischk.]	Px	seneciphylline	413
Senecio ampullaceus Hook.	-	retrorsine	270,381
Senecio aquaticus Hill	-	seneciphylline	382
Senecio bipinnatisectus Belcher	Px,Rt	retrorsine	383
Senecio brasiliensis (Spreng.) Less. [*S. ambrosioides* Mart. ex Baker]	-	jacobine	270
Senecio brasiliensis var. *brasiliensis*	Pl	retrorsine	385
Senecio bupleuroides DC. [*S. glaberrimus* DC.]	-	retrorsine	270,381,399
Senecio cannabifolius Less. [*S. palmatus* Pall. ex Ledeb.]	Pl	seneciphylline	380,382
Senecio chrysanthemoides DC.	Rt	seneciphylline	388
Senecio chrysocoma Meerb. [*S. graminifolius* Jacq.]	-	retrorsine	400
Senecio cineraria DC.	Lf,St	jacobine	270,381,390,392
	-	retrorsine	390
	-	seneciphylline	391,392
	-	seneciphylline	393
Senecio desfontainei Druce	-	seneciphylline	394
Senecio discolor (Sw.) DC.	Lf	isatidine	394
	Lf,St	retrorsine	394,395
	-	seneciphylline	395
Senecio douglasii DC.	-	retronecine	396
Senecio douglasii DC. var. *longilobus* (Benth.) L. Benson [*S. longilobus* Benth.]	-	retrorsine	270
Senecio eremophilus Richards.	-	retrorsine	270,381
Senecio erraticus var. *barbariifolius*	Fl,Lf	seneciphylline	397
Senecio erucifolius L.	-	seneciphylline	398
Senecio fremontii Torr. & Gray	-	seneciphylline	270,390
Senecio grandifolius Less.	Lf,St	seneciphylline	401
Senecio grisebachii Baker	-	retrorsine	402
Senecio incanus L. ssp. *carniolicus* (Willd.) Br.-Bl.	-	seneciphylline	380
	-	seneciphylline	390
Senecio ilicifolius L.	-	retrorsine	403-405

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Senecio isatideus DC	-	isatidine	399,406
	-	retrorsine	399,404,407
Senecio jacobaea L.	Px	jacobine	476,391,408-412
	Pl	seneciphylline	391,411,412
Senecio junceus (Less.)Harv.	-	seneciphylline	390
Senecio kubensis Grossh.	Px,Rt	seneciphylline	414
Senecio lampsanoides DC.	Pl	seneciphylline	415
Senecio latifolius DC. [*S. retroreus* DC.]	-	isatidine	407
Senecio latifolius DC. [*S. sceleratus* Schweikerdt]	-	retrorsine	381,400
Senecio longiflorus[a]	-	seneciphylline	382,390
Senecio othonnae Bieb.	Pl	seneciphylline	416
Senecio paucicalyculatus Klatt	-	isatidine	417
	-	retrorsine	390
Senecio paucifolius S.G.Gmel.	Pl	seneciphylline	391
Senecio praealtus Bertol. var. *borysthenicus* Andrz. ex DC. [*S. borysthenicus* Andrz. ex Bess.]	Rt	seneciphylline	384,385
Senecio propinquus Schischk.	Rt,Rz	seneciphylline	422
Senecio pterophorus DC.	-	retrorsine	400,403
	-	seneciphylline	270
Senecio quadridentatus Labill. [*Erechtites quadridentata* (Labill.)DC.]	Pl	retrorsine	377,378
	-	seneciphylline	377,378
Senecio racemosus (Bieb.)DC.	Rt,Rz	seneciphylline	423
Senecio renardii Winkl.	-	seneciphylline	270
Senecio riddellii Torr. & Gray	-	retrorsine	270
Senecio ruderalis Harv.	-	retrorsine	424
Senecio spartioides Torr. & Gray	-	seneciphylline	425
Senecio subalpinus Koch	Lf	seneciphylline	426
Senecio swaziensis Compton	-	retrorsine	427
Senecio venosus Harv.	-	retrorsine	381,400
Senecio viminalis Bremek.	-	retrorsine	382,390
Senecio vulgaris L.	Pl,Rz	seneciphylline	428,430
DIPSACACEAE			
Succisa pratensis Moench. [*Scabiosa succisa* L.]	-	sanguinarine	273
FAGACEAE			
Castanea sativa Mill.	Lf	tannins	167
Quercus aegilops L.	Lf	tannins	167

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
KRAMERIACEAE			
Krameria ixina L.	Px	tannins	170-173
Krameria triandra Ruiz & Pav.	Rt	tannins	173
LABIATAE			
Ocimum basilicum L.	Vo	safrole	431
LAURACEAE			
Cinnamomum bodinieri Leveille	Vo	safrole	432
Cinnamomum camphora (L.) J.Presl	Lf,Vo	safrole	433-437
Cinnamomum camphora var. *glaucescens* A.Br.	Lf	safrole	438
Cinnamomum doederleinii Engl.	Vo	safrole	439
Cinnamomum massoia Schewe	Bk	safrole	440
Cinnamomum micranthum (Hayata) Hayata	Vo	safrole	441
Cinnamomum parthenoxylon (Jack) Meissn.	Wd	safrole	442
Licaria puchury-major (Mart.) Kosterm.	Sd,Tw	safrole	443-444
Nectandra falcifolia (Nees) Castiglioni	-	safrole	445
Ocotea cymbarum[a]	-	safrole	446
Ocotea pretiosa (Mart.) Mez	Vo,Wd	safrole	447-449
Sassafras albidum (Nutt.) Nees	Vo	safrole	450
LEGUMINOSAE			
Acacia pubescens (Vent.)R.Br. [*A. mollissima* Willd.]	Lf	tannins	167
Acacia villosa (Sw.) Willd.	Lf	tannins	171-173
Ammodendron viscosus[a]	Lf	sanguinarine	382
Crotalaria crispata F.Muell. ex Benth.	Pl	monocrotaline	382
Crotalaria grahamiana Wight & Arn.	Sd	monocrotaline	452,453
Crotalaria mysorensis Roth.	-	monocrotaline	455
Crotalaria recta Steud. ex A.Rich.	-	monocrotaline	382
Crotalaria retusa L.	-	monocrotaline	456
Crotalaria sagittalis L.	Fr	monocrotaline	457
Crotalaria sericea Retz. [*C. leschenaultii* DC.]	Fr	monocrotaline	382,454
Crotalaria spartioides DC.	Pl	retrorsine	458
Crotalaria spectabilis Roth.	-	monocrotaline	196,459
Derris chinensis Benth.	-	rotenone	460
Derris elliptica (Wall.)Benth	Rt	rotenone	460-471
Derris ferruginea (Roxb.) Benth.	-	rotenone	468
Derris malaccensis Prain	Rt,Sd	rotenone	470,472
Derris trifoliata Lour. [*D. uliginosa* (Willd.)Benth.]	Sd	rotenone	472
Lonchocarpus nicou (Aubl.)DC.	-	rotenone	460,473
Lonchocarpus urucu Killip & A.C.Sm.	-	rotenone	473
Lonchocarpus utilis A.C.Sm.	Rt	rotenone	476
Milletia ferruginea (Hochst.) Baker	Sd	rotenone	477
Milletia pachycarpa Benth.	Rt	rotenone	478

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Milletia taiwaniana (Hayata) Hayata	Rt	rotenone	479
Mundulea sericea (Willd.) A.Cheval.	Bk	rotenone	472
Neorautanenia ficifolia (Benth.) C.A.Sm.	Tu	rotenone	474
Pachyrhizus erosus (L.) Urban	Sd	rotenone	475
Piscidia piscipula (L.) Sarg. [*P. erythrina* L.]	Bk,Rb,Rt	rotenone	480
Robinia viscosa Vent.	see *Ammodendron viscosus*		
Tephrosia candida DC.	Rb,Sd	rotenone	472,481
Tephrosia obovata Merrill	Pl	rotenone	482
Tephrosia piscatoria (Dryand.)Pers.	-	rotenone	460
Tephrosia purpurea (L.) Pers.	Rb,Sd	rotenone	481
Tephrosia sinapou (Buchoz) A.Cheval [*T. toxicaria* (Sw.)Pers.]	Rt	rotenone	469,472,487
Tephrosia villosa (L.)Pers. subsp. *ehrenbergiana* (Schweinf.) Brammitt [*T. ehrenbergiana* Schweinf.]	Sd	rotenone	472
Tephrosia virginiana (L.) Pers. [*Cracca virginiana* L.]	Rt	rotenone	451,483-486
Tephrosia vogelii Hook.fil.	Lf,Pl,Rt,Sd,St	rotenone	472
LINACEAE			
Linum album Kotschy ex Boiss.	Pl	podophyllotoxin	488
LOGANIACEAE			
Strychnos pseudo-quina St.-Hil.	Bk,Wd	quinine	489
MAGNOLIACEAE			
Magnolia salicifolia (Sieb. & Zucc.) Maxim.	Bd	safrole	490
MONIMIACEAE			
Laurelia philippiana Looser [*L. serrata* Phil.]	Lf,St	safrole	491-493
MYOPORACEAE			
Eremophila longifolia (R.Br.) F.Muell.	Lf	safrole	493
MYRISTICACEAE			
Myristica fragrans Houtt.	Vo	safrole	494-496
MYRTACEAE			
Eucalyptus redunca Schauer	Lf	tannins	167
PAPAVERACEAE			
Argemone albiflora Hornem. subsp. *albiflora* [*A. alba* Lestib.]	Px,Rt	sanguinarine	497
Argemone mexicana L.	Rt,Sd	sanguinarine	200,206,501
Argemone platyceras Link & Otto	Px,Rt	sanguinarine	502
Argemone polyanthemos (Fedde) G.Ownby	Pl	sanguinarine	503
Argemone subfusiformis G.Ownby	Px	sanguinarine	504
Bocconia frutescens L.	Lf	sanguinarine	273
Bocconia latisepala Wats.	Bk,Lf,Sd,St	sanguinarine	506
Bocconia pearcei Hutchins.	Rt,St	sanguinarine	510
Chelidonium majus L.	La,Pl,Rt	sanguinarine	511-524

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Corydalis cheilanthifolia Hemsl.	Bk,Lf,St	sanguinarine	382
Corydalis incisa (Thunb.)Pers	-	sanguinarine	525
Corydalis ledebouriana Kar. & Kir.	-	sanguinarine	526,527
Corydalis persica Cham. & Schlecht.	Ca	sanguinarine	528
Corydalis sempervirens (L.)Pers [*C. glauca* Pursh]	Bk,Lf,Sd,St	sanguinarine	382
Corydalis sewerzowii Regel	-	sanguinarine	526
Corydalis stricta Stephen ex DC.	Rt	sanguinarine	529
Corydalis vaginans Royle	-	sanguinarine	530
Dicentra cucullaria (L.)Bernh.	-	sanguinarine	380
Dicentra formosa (Torr. & Gray) Walp.	Lf,St	sanguinarine	382
Dicentra spectabilis (L.) Lem.	-	sanguinarine	531
Dicranostigma franchetianum (Prain) Fedde	Px,Rt	sanguinarine	532
Dicranostigma lactucoides Hook.fil. & Thoms.	Bk,Pl,Px,Sd	sanguinarine	573
Eschscholzia californica Cham. [*E. glauca* Greene]	Lf,Rt,St	sanguinarine	576
Eschscholzia lobbii Greene	-	sanguinarine	576
Eschscholzia oregana Greene	Bk,Lf,Pl,Rt,Sd	sanguinarine	576
Fumaria densiflora DC. [*F. micrantha* Lag.]	Lf,Sd,St	sanguinarine	382
Fumaria indica (Hausskn.) Pugsley	Bk,Rt,Sd,St	sanguinarine	382
Fumaria officinalis L.	Lf,Rt,Sd,St	sanguinarine	382
Glaucium corniculatum (L.) Rudolph	-	sanguinarine	380
Glaucium elegans Fisch. & Meyer	Rt	sanguinarine	577
Glaucium fimbrilligerum Trautv.)Boiss [*G. vitellinum* B.Fedtsch.]	Px	sanguinarine	512
Glaucium flavum Crantz	Rt	sanguinarine	382
Glaucium flavum var.*fulvum*(Smith) Fedde.	Rt	sanguinarine	598
Glaucium leiocarpum Boiss. [*G. flavum* var. *leiocarpum*]	Rt	sanguinarine	599
Glaucium oxylobium Boiss. & Buhse	-	sanguinarine	382
Glaucium squamigerum Kar. & Kir.	Pl	sanguinarine	532
Hunnemannia fumariifolia Sweet	-	sanguinarine	533
Hylomecon vernalis Maxim.	-	sanguinarine	380
Hypecoum imberbe Sibth. & Sm. [*H. grandiflorum* Benth.]	Bk,Lf,Sd	sanguinarine	382
Hypecoum leptocarpum Hook.fil. & Thoms.	Pl	sanguinarine	534
Hypecoum pendulum L. var. *trilobum* (Trautv.)Cullen [*H. trilobum* Trautv.]	-	sanguinarine	380
Hypecoum procumbens L.	Pl	sanguinarine	534
Macleaya cordata (Willd.)R.Br. [*Bocconia cordata* Willd.]	Lf,Sd	sanguinarine	505,535-538
Macleaya microcarpa (Maxim.) Fedde [*Bocconia microcarpa* Maxim.]	Lf,Px,Rt,St	sanguinarine	507-509,535-538
Meconopsis aculeata Royle	-	sanguinarine	382
Meconopsis betonicifolia Franchi	Pl	sanguinarine	382
Meconopsis cambrica (L.) L.Vig.	-	sanguinarine	382

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Meconopsis dhwojii G.Taylor	Lf,St	sanguinarine	382
Meconopsis horridula (Prain) Prain [*M. rudis* (Prain) Prain]	Rt	sanguinarine	382,539
Meconopsis latifolia (Prain) Prain	-	sanguinarine	382
Meconopsis paniculata (D.Don) Prain	-	sanguinarine	382
Papaver aculeatum Thunb.	Pl	sanguinarine	382
Papaver alpinum L.	Pl	sanguinarine	382
Papaver alpinum subsp. *alpinum* [*P. alpinum* subsp. *burseri* (Crantz)Fedde]	Pl	sanguinarine	382
Papaver alpinum var. *kerneri* (Hayek)Fedde	Pl	sanguinarine	382
Papaver alpinum var. *tatricum* A.Nyarady	Pl	sanguinarine	382
Papaver anomalum Fedde	Pl	sanguinarine	382
Papaver armeniacum (L.)DC	Pl	sanguinarine	382
Papaver atlanticum	-	sanguinarine	380
Papaver commutatum Fisch. & Mey.	Lf	sanguinarine	382
Papaver dubium L.	Lf	sanguinarine	382
Papaver fugax Poir. [*P. caucaeicum* Bieb.]	-	sanguinarine	540
Papaver glaucum Boiss. & Hausskn.	Pl,Sd	sanguinarine	541
Papaver hybridum L.	Rt	sanguinarine	382
Papaver monanthum Trautv.	Bk	sanguinarine	382
Papaver nudicaule L.	Bk,Lf,Rt	sanguinarine	382
Papaver nudicaule subsp. *nudicaule* [*P. nudicaule* var. *leiocarpum* (Turcz.)Turcz]	Pl	sanguinarine	382
Papaver nudicaule subsp. *nudicaule* [*P. nudicaule* subsp. *xanthopetalum* Trautv.Fedde]	Pl	sanguinarine	382
Papaver oreophilum Rupr.	Pl	sanguinarine	382
Papaver orientale L.	-	sanguinarine	380
Papaver pavoninum[a]	-	sanguinarine	380
Papaver pilosum Sibth. & Sm.	Lf,Rt,St	sanguinarine	382
Papaver polychaetum Schott & Kotschy	-	sanguinarine	542
Papaver pseudocanescens Popov	Pl	sanguinarine	382
Papaver radicatum Rottb.	Pl,Sd	sanguinarine	543
Papaver rhoeas L.	Pl	sanguinarine	380
Papaver rubro-aurantiacum (Fisch. ex DC.) Lundstr. [*P. nudicaule* var. *rubro-aurantiacum* Fisch. ex DC.]	Pl	sanguinarine	382
Papaver somniferum L. [*P. setigerum* DC.]	-	sanguinarine	380
Papaver suaveolens Lapeyr.	Pl	sanguinarine	382
Papaver tauricum[a]	Pl	sanguinarine	382
Papaver triniifolium Boiss.	Pl	sanguinarine	542,544
Platystemon californicus Benth.	-	sanguinarine	380,545
Romneya coulteri Harv.	Rt,St	sanguinarine	382
Sanguinaria canadensis L.	Rt	sanguinarine	497,548,577

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Stylomecon heterophylla (Benth.) G.Taylor	-	sanguinarine	382
Stylophorum diphyllum (Michx.) Nutt.	Lf,Rt,St	sanguinarine	549
PIPERACEAE			
Piper nigrum L.	Vo	safrole	550
ROSACEAE			
Sorbus aucuparia L.	Fr	parasorbic acid	12
RUBIACEAE			
Cinchona carabayensis Wedd. [*C. hasskarliana* Miq.]	Bk	quinine	273
Cinchona humboldtiana Lamb.	Bk	quinine	273,556
Cinchona ledgeriana (Howard) Moens et Trimen	Bk,Hw,Lf,Rt,Sd,St	quinine	554-555
Cinchona micrantha Ruiz & Pav	Bk	quinine	556
Cinchona obaldiana Klotzsch	Bk	quinine	273
Cinchona oblongifolia[a]	Bk	quinine	273
Cinchona officinalis L. [*C. calisaya* Wedd.]	Bk	quinine	551,555
Cinchona officinalis L. [*C. calisaya* var. *josephiana* Wedd.]	Bk	quinine	551
Cinchona officinalis L. [*C. condaminea* Humb. & Bonpl.]	Bk	quinine	273
Cinchona officinalis L. [*C. lancifolia* Mutis]	Bk	quinine	273
Cinchona officinalis L. [*C. lucumifolia* Pav. ex Lindl.]	Bk	quinine	273
Cinchona officinalis L. [*C. macrocalyx* DC.]	Bk	quinine	555
Cinchona pitayensis Wedd. [*C. corymbosa* Karst.]	Bk	quinine	273
Cinchona pubescens Vahl [*C. rufinervis* Wedd.]	Bk	quinine	273
Cinchona pubescens Vahl [*C. scrobiculata* Humb. & Bonpl.]	Bk	quinine	273
Cinchona pubescens Vahl [*C. succirubra* Pav. ex Klotzsch]	Bk	quinine	551,553,555
Cinchona tucujensis Karst.	Bk	quinine	273
Hintonia latiflora (Sesse & Moc. ex DC.) Bullock [*Coutarea latiflora* Sesse & Moc. ex DC.]	Bk	quinine	273
Ladenbergia magnifolia (Ruiz & Pav.) Klotzsch [*Cinchona nitida* Benth.]	Bk	quinine	273
Remijia bicolorata Hodgkin	Bk	quinine	273
Remijia pedunculata[a]	Bk	quinine	273
Remijia purdieana Wedd.	Bk	quinine	273
RUTACEAE			
Zieria smithii Andrews	Br,Lf	safrole	557
SIMAROUBACEAE			
Picrolemma pseudocoffea Ducke	St	quinine	489
SOLANACEAE			
Nicotiana tabacum L.	Lf	N'nitrosonornicotine	13

Table 8.--The Distribution of Selected Major Oncogens in the Plant Kingdom--continued

Plant name	Part[b]	Oncogen	Ref.
Solanum incanum L.	Fr	dimethylnitrosamine	217
THEACEAE			
Camellia sinensis (L.) O.Kuntze	Lf		183-185
UMBELLIFERAE			
Centella asiatica (L.) Urban [*Hydrocotyle asiatica* L.]	Pl,St	asiaticoside	558-563,564-565

[a]The validity of this binomial could not be verified in the current literature.

[b]Key for plant parts: Bd, bud; Bk, bark; Br, branch; Bu, bulb; Ca, capsule; Co, corm; En, endosperm; Fd, frond; Fl, flower; Fr, fruit; Hs,seed hulls ; Hw, heartwood; Kn, kernel; La, latex; Lf, leaf; Nt, nut; Pc, pericarp; Pl, whole plant; Px, aerial parts; Rb, rootbark; Rt, root; Rz, rhizome; Sd, seed; Sl,strobil; Sn,nut outer shell;SO, seed oil; St, stem; Tu, tuber; Us,unhulled seed ; Vo, volatile oil; Wd, wood; Ws, stem wood.

TUMOR-PROMOTING AGENTS AND IRRITANTS FROM THE EUPHORBIACEAE

Croton tiglium L.

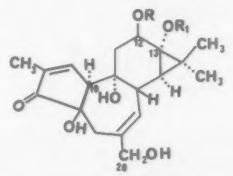
Croton oil, derived from the seeds of *Croton tiglium*, was first reported to be a tumor promoter by Berenblum (566) in 1941. He found that when it was used in conjunction with a very dilute solution of benzpyrene in acetone, a marked augmentation of tumor induction resulted. A resin obtained from croton oil was shown to give a more pronounced effect. Berenblum noted, however, that neither croton oil, nor the resin from it, augmented the oncogenic action of benzpyrene.

In a later study (567), he reported that treatment of an area of skin with benzpyrene, followed by croton resin, caused a strikingly higher rate of tumors than did treatment with benzpyrene alone. Application of croton resin prior to benzpyrene treatment, however, did not lead to tumor promotion (567). It was also observed that following application of croton resin to papillomas, conversion of the latter to malignant tumors was facilitated (567). The work of Berenblum (566) eventually became the basis of a popular, standard test method for tumor-promoting substances. This test, which employs one subminimal dose of a suspected carcinogen followed by repeated application of croton oil, has served as a tool for analyzing the biologic aspects of carcinogenic processes (5).

The active principles of *Croton tiglium* eluded identification for several years. In 1964, Hecker and co-workers (568) identified the major tumor-promoting constituent in croton oil as 12-tetradecanoylphorbol-13-acetate (phorbol myristate acetate) (XLV) (fig 9). Since that time, a number of other minor phorbol derivatives have been isolated from this plant. Although many of these have been shown to elicit tumor-promoting activity, they are less active than XLV (3–5).

Fürstenberger and Hecker (4) have concluded that the tumor-promoting diterpenes of the Euphorbiaceae and Thymelaeaceae are all phorbol esters, which must have a free hydroxyl function at the 20-position and a 4β,10α configuration at the junction of the five- and seven-membered rings (fig 9).

In addition to several phorbol esters (eg, 12-*O*-tiglyl-phorbol-13-butyroate, 12-*O*-tiglyl-phorbol-13-dodecanoate, and 12-*O*-butyroyl-phorbol dodecanoate [569]) showing tumor-promoting activity, the parent alcohol phorbol (XLVI) also acts as an initiator of dimethylnitrosamine oncogenesis (570). The tumor-promoting ability of phorbol esters has been shown to correlate with their ability to induce a sustained stimulation of macromolecular synthesis in mouse epidermis (571). Raick (572), after studying the effects of 12-*O*-tetradecanoyl-phorbol-13-acetate (XLV) on mouse epidermis, has suggested that tumor promoters might act by altering the normal differentiation pathway, thereby enabling cells that have been initiated by a subthreshold dose of car-



	R	R_1
XLV	$-CO(CH_2)_{12}-CH_3$	$-COCH_3$
XLVI	-H	-H

XLV 12-Tetradecanoyl-phorbol-13-acetate (phorbol myristate acetate)

XLVI Phorbol

FIGURE 9.—Structures of *Croton tiglium* cocarcinogens.

cinogen to manifest their oncogenic phenotype. Phorbol myristate acetate (XLV) is now widely used as a tumor promoter in studying carcinogenic processes (4,573–577).

Euphorbia and Related Euphorbiaceae as Tumor-Promoters

A number of irritant and/or tumor-promoting phorbol and/or 12-deoxyphorbol esters have been isolated from the oil or latex of *Euphorbia cooperi* N.E.Br. (578), *E. ingens* E.Mey. ex Boiss. (3,5), *E. lathyris* L. (3,5), *E. resinifera* Berg (579,580), *E. unispina* N.E.Br. (580), *E. tirucalli* L. (4), *E. triangularis* Desf. (581), and *E. fortissima* Leach (582). Irritant and/or tumor-promoting esters of 12-deoxyphorbol and 12-deoxy-16-hydroxyphorbol have also been isolated from *E. cooperi* (569) and *E. triangularis* (569). That these esters are irritants does not necessarily mean that they are also tumor-promoters. Indeed, most of them do not have the latter activity (3–5,569,579–582). Many diterpene esters have been isolated from *Euphorbia* species and a number of them possess the structural characteristics thought to be required for tumor-promoting activity (583–585); however, they have not yet been evaluated in this regard.

Huratoxin (XLVII) (fig 10) and two related diterpene esters have recently been isolated from the toxic plant *Hippomane mancinella* L. (586). Huratoxin has also been isolated from *Hura crepitans* L. (587). Hydrophilic extracts from the latex of this plant had irritant and tumor-promoting activity, but the isolated irritant diterpenes were not reported to have tumor-promoting activity.

Similar phorbol esters have also been isolated from *Aleurites fordii* Hemsl. and were shown to be piscicidal, but tumor-promoting activity was not reported (588).

Tumor-Promoters From the Thymelaeaceae

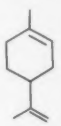
Mezerein (XLIX) (589–592) and daphnetoxin (XLVIII) (592,593) (fig 10), which are known tumor-promoters, have been isolated from *Daphne mezereum* L. Both of these compounds also have antileukemic activity in mice (593).

Tumor-Promoting Citrus Oils

In 1959, Roe (594) reported that the oil of sweet orange had tumor-promoting activity when it was applied weekly to the skin of mice that had been painted 3 weeks before with 9,10-dimethyl-1,2-benzanthracene (DMBA). Roe and Pierce (595) subsequently expanded this study, showing that the oils derived from the peel of four citrus fruits, sweet orange, lemon, grapefruit, and lime, caused epidermal hyperplasia in mice. All of the oils also had a tumor-promoting effect when applied weekly to mouse skin beginning 3 weeks after a single application of DMBA.

The effects of orange oil have been studied in greatest detail (595). In contrast to the good tumor-promoting activity shown by orange oil when it was applied to mouse skin following topically applied DMBA, orange oil showed only weak activity following either topical or ip administration of urethane, and no activity when injected intradermally following ip administration of urethane. Orange oil did not induce tumors when injected intradermally or when applied to the skin undiluted or diluted with acetone. Although 80% orange oil in acetone was as effective a tumor promoter as undiluted orange oil, only weak activity was shown by 40% orange oil.

Roe and Pierce (595) tested the terpene and nonterpene fractions of orange oil on the skin of DMBA-treated mice and found that the tumor-promoting activity was associated with the terpene fraction. The authors suggested, furthermore, that the active component might be the terpene, *d*-limonene (L).



L

Lime oil (596,597), heated lime oil (597), orange oil (597), and *d*-limonene (597) were tested for tumor-promoting activity on the fore-stomach epithelium of mice; the initiator (DMBA or benzo[a]pyrene) and these potential tumor promoters were administered by stomach tube. Lime oil (596,597), heated lime oil (597), and orange oil (597) were active; orange oil, however, "promoted" tumor formation even in the absence of prior treatment with an initiator, ie, after prior treatment with only the polyethylene glycol solvent. Results with *d*-limonene in these studies were equivocal (597).

Field and Roe (597) pointed out that at about the time these experiments were being carried out, the per capita consumption of citrus oils in Britain over a 40-year period averaged 600–700 ml, whereas on the basis of comparative body weight and lifespan, the dose being given to mice in their tumor promotion experiments was about six times that amount. They concluded, therefore, that it was highly unlikely that the citrus oils were contributing to the incidence of human cancer in Britain. Nevertheless, they did indicate that the consumption of citrus

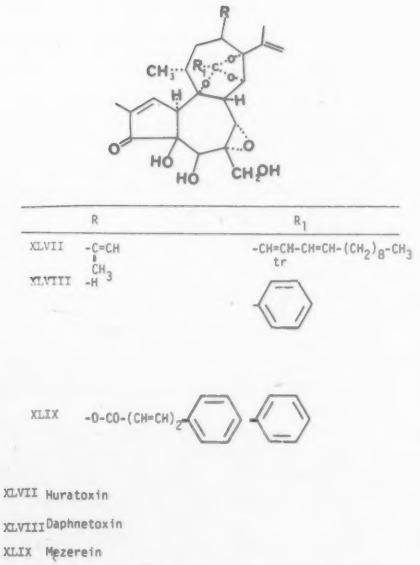


FIGURE 10.—Miscellaneous cocarcinogens from plants.

drinks made from the entire pulverized fruit could lead to an increased intake of citrus oils.

CONCLUSIONS

Although only 28 plants (whole or as extracts) have been shown to induce malignant tumors in laboratory animals, some 28 plant principles of known structure are known to be reproducibly oncogenic in animals. These 28 compounds represent alkaloids (pyrrolizidine, indole, tropolone, quinoline, purine, and benzophenanthridine types), nitroso derivatives, triterpene glycosides, lignans, isoflavans, allyl benzenoids, simple α-pyrenes, and carbocyclic hydroxy acids. A literature search has revealed that these compounds have been identified in at least 454 species of plants, classified in 110 genera and 34 families of Spermatophyta and Pteridophyta. Thus, these 454 species are all potentially oncogenic.

Tumor-promoters (cocarcinogens) have been identified in at least 12 species of higher plants, all classified in the Euphorbiaceae or Thymelaeaceae. A majority of tumor-promoting compounds are esters of the diterpene phorbol. Phorbol-type diterpenes represent a large group of natural products with a fairly restricted taxonomic distribution, and many compounds of this type have not been evaluated for tumor-promoting or oncogenic properties.

REFERENCES

1. PLOWRIGHT W, LINSELL CA, and PEERS FG. A focus of rumenal cancer in Kenyan cattle. Br J Cancer 25:72–80, 1971.
2. FARNSWORTH NR, BLOMSTER RN, MESSMER WM, ET AL. A phytochemical and biological review of the genus *Croton*. Lloydia 32:1–28, 1969.
3. HECKER E. Cocarcinogene Wirkstoffe aus Euphorbiaceen. Planta Med 16(suppl):24–45, 1968.
4. FÜRSTENBERGER G, and HECKER E. Zum Wirkungsmechanismus cocarcinogener Pflanzeninhaltstoffe. Planta Med 22:241–266, 1972.
5. HECKER E. Cocarcinogenic principles from the seed oil of *Croton tiglium* and from other Euphorbiaceae. Cancer Res 28:2338–2349, 1968.
6. SCHOENTAL R. Toxicology and carcinogenic action of pyrrolizidine alkaloids. Cancer Res 28:2237–2246, 1968.
7. WHITING MG. Toxicity of cycads. Econ Bot 17:270–302, 1963.
8. WHITING MG, ed. Proceedings of the 4th Conference on the Toxicity of Cycads. Washington, DC, US Department of Health, Education, and Welfare, US Public Health Service, 1967.
9. WHITING MG, SPATZ M, and MATSUMOTO H. Research progress on cycads. Econ Bot 20:98–102, 1966.
10. YANG MG, and MICKELSEN O. Cycads. *In* Toxic Constituents of Plant Foodstuffs (Liener IF, ed). New York, Academic Press, 1969, pp 159–167.
11. YANG MG, KOBAYASHI A, and MICKELSEN O. Bibliography of cycad research. Fed Proc 31:1543–1546, 1972.
12. PREUSSMANN R. Carcinogene Pflanzeninhaltsstoffe. Planta Med 22:217–228, 1972.
13. HOFFMANN D, HECHT SS, ORNAF RM, ET AL. *N'*-nitrosonornicotine in tobacco. Science 180:265–266, 1974.
14. JOHNSON DE, and RHOADES JW. *N*-nitrosamines in smoke condensate from several varieties of tobacco. J Natl Cancer Inst 48:1845–1847, 1972.
15. ARCOS JC, ARGUS MF, and WOLF G. Chemical Induction of Cancer. New York, Academic Press, 1968, vol 1, pp 340–457.
16. MAGEE PN. *In* Methods in Toxicology (Paget GE, ed). Philadelphia, F. A. Davis Co, 1970, pp 158–184.
17. WEISBURGER EK. A critical evaluation of the methods used for determining carcinogenicity. J Clin Pharmacol 15:5–15, 1975.
18. WEISBURGER J, and WEISBURGER E. *In* Methods in Cancer Research (Busch H, ed). New York, Academic Press, 1967, vol 1, pp 307–386.
19. GRASSO P. Carcinogenicity testing and permitted lists. Chem Br 6:17–22, 1970.
20. RALL DP. Candidate drugs for carcinogenic property studies: procedures. J Clin Pharmacol 15:1–4, 1975.
21. AMES BN, DURSTON WE, YAMASAKI E, ET AL. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285, 1973.
22. ZEDECK MS, STERNBERG SS, YATAGANAS X, ET AL. Early changes induced in rat liver by methylazoxymethanol acetate: mitotic abnormalities and polyploidy. J Natl Cancer Inst 53:719–723, 1974.
23. FARBER E. Mechanisms by which chemicals initiate cancer. J Clin Pharmacol 15:24–28, 1975.
24. NIGRO ND, SINGH DV, CAMPBELL RL, ET AL. Effect of dietary beef fat on intestinal tumor formation by azoxymethane in rats. J Natl Cancer Inst 54:439–442, 1975.
25. ADELAER TF, and MELDAL-JOHNSEN CM. Bracken poisoning in cattle in the Natal midlands. J S Afr Vet Assoc 39:91–99, 1968.
26. EVERS RA, and LINK RP. Poisonous plants of the midwest.

Special Publication 24. Urbana, University of Illinois at Urbana-Champaign, College of Agriculture, 1972.

27. EVANS IA. The radiomimetic nature of bracken toxin. Cancer Res 28:2252–2261, 1968.
28. EVANS WC. Bracken poisoning of farm animals. Vet Rec 76:367–369, 1964.
29. EVANS WC, EVANS ETR, and HUGHES LE. Studies on bracken poisoning in cattle. I. Br Vet J 110:205–206, 1954.
30. EVANS WC, EVANS ETR, and HUGHES LE. Studies on bracken poisoning in cattle. II. 1950 Bracken poisoning experiments (Lluest Farm). Br Vet J 110:365–380, 1954.
31. EVANS WC, EVANS ETR, and HUGHES LE. Studies on bracken poisoning in cattle. III. Field outbreaks of bovine poisoning. Br Vet J 110:426–442, 1954.
32. EVANS WC, EVANS IA, THOMAS AJ, ET AL. Studies on bracken poisoning in cattle. IV. Br Vet J 114:180–198, 1958.
33. EVANS WC, EVANS JA, CHAMBERLAIN AG, ET AL. Studies on bracken poisoning in cattle. VI. Br Vet J 115:83–85, 1959.
34. EVANS WC, JONES NR, and EVANS RA. The mechanism of the anti-aneurin activity of bracken (*Pteris aquilina*). Biochem J 46:38, 1950.
35. PAMUKCU AM. Investigations on the pathology of enzootic bovine haematuria in Turkey. Zentralbl Veterinaermed [A] 2:409–429, 1955.
36. PAMUKCU AM. Tumors of the urinary bladder in cattle and water buffalo affected with enzootic bovine hematuria. Zentralbl Veterinaermed [A] 4:185–197, 1957.
37. GEORGIEV R, ANTONOV S, VRIGASOV A, ET AL. Uber die Aetiologie der chronischen vesikalen Hämaturie der Rinder. I. Zur Frage des Karzinomatosen Charakter der chronischen vesikalen Hämaturie der Rinder. Wien Tieraerztl Monatsschr 51:641–657, 1964.
38. GEORGIEV R, VRIGASOV A, ANTONOV S, ET AL. Versuche zur Feststellung der Anwesenheit Kanzerogener stoffe im Harn der mit Heu aus Hämaturie-gebieten gefutterten Kuhe. Wien Tieraerztl Monatsschr 50:589–595, 1963.
39. GEORGIEV R, and ANTONOV S. Über die Aetiologie der chronischen vesikalen Hämaturie der Rinder. II. Versuche zur Feststellung der Anwesenheit Kanzerogener Metaboliten im Harn gesunder Kühe aus einem Hämaturiefreien Gebiet bei Fütterung mit Heu aus Hämaturie-gebieten. Wien Tieraerztl Monatsschr 52:90–94, 1965.
40. PAMUKCU AM. Tumors of the urinary bladder in cattle with special reference to etiology and histogenesis. Acta Un Int Cancr 18:625–638, 1962.
41. PAMUKCU AM. Epidemiologic studies on urinary bladder tumors in Turkish cattle. Ann NY Acad Sci 108:938–947, 1963.
42. PAMUKCU AM, GOKSOY ST, and PRICE JM. Urinary bladder neoplasms induced by feeding bracken fern (*Pteris aquilina*) to cows. Cancer Res 27:917–924, 1967.
43. ROSENBERGER G, and HEESCHEN W. Alderfarn (*Pteris aquilina*) die Ursache des sog. Stallrotes der Rinder (Haematurie vesicalis bovis chronica). Dtsch Tieraerztl Wochenschr 67:201–208, 1960.
44. EVANS JA, and MASON J. Carcinogenic activity of bracken. Nature 180:913–914, 1965.
45. SCHACHAM P, PHILP RB, and GOWDEY CW. Antihematopoietic and carcinogenic effects of bracken fern (*Pteridium aquilinum*) in rats. Am J Vet Res 31:191–197, 1970.
46. HIRONO I, SHIBUYA C, FUSHIMI K, ET AL. Studies on carcinogenic properties of bracken, *Pteridium aquilinum*. J Natl Cancer Inst 45:179–188, 1970.
47. PRICE JM, and PAMUKCU AM. The induction of neoplasms of the urinary bladder of the cow and the small intestine of the rat by feeding bracken fern (*Pteris aquilina*). Cancer Res 28:2247–2251, 1968.
48. PAMUKCU AM, OLSON C, and PRICE JM. Assay of fractions of bovine urine for carcinogenic activity after feeding bracken fern (*Pteris aquilina*). Cancer Res 26:1745–1753, 1966.
49. PAMUKCU AM, PRICE JM, and BRYAN GT. Assay of fractions of bracken fern (*Pteris aquilina*) for carcinogenic activity. Cancer Res 30:902–905, 1970.
50. EVANS IA, and WIDDOP B. Carcinogenic activity of bracken. *In* British Empire Cancer Campaign for Research (Annual Report). Eastbourne, UK, Sumfield and Day, Ltd, 1966, part 2, p 377.
51. HIRONO C, SHIMIZU M, and FUSHIMI K. Carcinogenic activity of processed bracken used as human food. J Natl Cancer Inst 48:1245–1249, 1972.
52. WANG CY, PAMUKCU AM, and BRYAN GT. Isolation of fumaric acid, succinic acid, astragalin, isoquercitrin and tiliroside from *Pteridium aquilinum*. Phytochemistry 12:2298–2299, 1973.
53. LEACH H, BARBER GD, EVANS IA, ET AL. Isolation of an active principle from the bracken fern that is mutagenic, carcinogenic, and lethal to mice on intraperitoneal injection. Biochem J 124:13P–14P, 1971.
54. GOKSOY K. Sigir mesane tümörlerinin tesekkülünde Egrelti obtunun (*Pteris aquilina*) rolü. PhD dissertation, Turkey, Ankara University, 1966.
55. PAMUKCU AM, and PRICE JM. Induction of intestinal and urinary bladder cancer in rats by feeding bracken fern (*Pteris aquilina*). J Natl Cancer Inst 43:275–279, 1969.
56. YALCINER S. Egrelti obtunum (*Pteris aquilina*) Kanserogenik etkisinin sicanlar uzerinde arastirilmasi. PhD dissertation, Turkey, Ankara University, 1969.
57. WIDDOP B. Separation and characterisation of various toxicological components of bracken. PhD dissertation, University of Wales, 1967.
58. HIRONO I, FUSHIMI K, MORI H, ET AL. Comparative study of carcinogenic activity in each part of bracken. J Natl Cancer Inst 50:1367–1371, 1973.
59. EVANS IA, and OSMAN MA. Carcinogenicity of bracken and shikimic acid. Nature 250:348–349, 1974.
60. YOSHIDA S, TAZAKI K, and MINAMIKAWA T. Occurrence of shikimic and quinic acids in Angiosperms. Phytochemistry 14:195–197, 1975.
61. Proceedings of the 3rd Conference on the Toxicity of Cycads, Chicago, Apr 1964 (sponsored by the National Institute of Neurological Diseases and Blindness, Bethesda, Md). Fed Proc 23:1333–1388, 1964.
62. Cycad research. *In* Report on the 4th Conference on Cycad Toxicity (sponsored by the National Institutes of Health, Bethesda, Md). Toxicon 3:171, 1965.
63. COOPER JM. Isolation of a toxic principle from the seeds of *Macrozamia spiralis*. J Proc R Soc N S Wales 74:450–545, 1941.
64. NISHIDA K, KOBAYASHI A, and NAGAHAMA T. Cycasin, a toxic glycoside of *Cycas revoluta*. I. Isolation and structure of cycasin. Bull Agric Chem Soc Jpn 19:77–84, 1955.
65. NISHIDA K, KOBAYASHI A, and NAGAHAMA T. Cycasin, a new toxic glycoside of *Cycas revoluta*. III. Isolation of cycasin from the stem of Japanese cycad. Seikagaku 28:70–74, 1956.
66. RIGGS NV. Glycosyloxyazoxymethane, a constituent of the seeds of *Cycas circinalis* L. Chem Ind, 1956, p 926.
67. NISHIDA K, KOBAYASHI A, NAGAHAMA T, ET AL. Some new azoxy glycosides of *Cycas revoluta*. I. Neocycasin A. Bull Agric Chem Soc Jpn 23:556–557, 1959.
68. NAGAHAMA T, NUMATA T, and NISHIDA K. Azoxy glycosides of *Cycas revoluta*. II. Neocycasin B and macrozamin. Bull Agric Chem Soc Jpn 23:556–557, 1959.
69. NAGAHAMA T, NISHIDA K, and NUMATA T. Azoxy glycosides of *Cycas revoluta*. VI. The structure of neocycasin C,

formed by transglycosylation with cycad emulsion. Bull Agric Chem Soc Jpn 24:536–537, 1960.
70. NAGAHAMA T. Neocycasins, new glycosides of cycads. Kagoshima daigaku Nogakubu Gakujutsu Hokoku 14:1–50, 1964 (*abstracted in* Chem Abstr 63:3312g, 1964).
71. NAGAHAMA T, NISHIDA K, and NUMATA T. Azoxy glycosides of *Cycas revoluta*. VII. Neocycasin E. Beta-cellobiosyloxyazoxymethane. Agric Biol Chem 25:937–938, 1961.
72. MATSUMOTO H, and STRONG FM. Occurrence of methylazoxymethanol in *Cycas circinalis*. Arch Biochem Biophys 101:299–310, 1963.
73. LAQUEUR GL, MICKELSEN O, WHITING M, ET AL. Carcinogenic properties of nuts from *Cycas circinalis* L. indigenous to Guam. J Natl Cancer Inst 31:919–951, 1963.
74. LAQUEUR GL. Carcinogenic effects of cycad meal and cycasin, methylazoxymethanol glycoside, in rats, and effects of cycasin in germ-free rats. Fed Proc 23:1387–1388, 1964.
75. SPATZ M. Carcinogenic effect of cycad meal in guinea pigs. Fed Proc 23:1384–1385, 1964.
76. HOCH-LIGETI C, MCDANIEL EG, and MATSUMOTO H. Cellular composition during tumor induction in rats by cycad husk. J Natl Cancer Inst 41:605–611, 1968.
77. YANG MG, SANGER VL, MICKELSEN O, ET AL. Carcinogenicity of long-term feeding of cycad husk to rats. Proc Soc Exp Biol Med 127:1171–1175, 1968.
78. LAQUEUR GL. Induction of intestinal neoplasms in rats with the glycoside, cycasin, and its aglycon. Arch Pathol Anat Physiol 340:151–163, 1965.
79. LAQUEUR GL, MCDANIEL EG, and MATSUMOTO H. Tumor induction in germ-free rats with methylazoxymethanol (MAM) and synthetic MAM acetate. (*Cycas circinalis*). J Natl Cancer Inst 39:355–371, 1967.
80. LAQUEUR GL. Gnotobiotes as useful tools in studies in carcinogenesis with cycasin, a natural glycoside. Adv Exp Med Biol 3:163–167, 1969.
81. SPATZ M. Toxic and carcinogenic alkylating agents from cycads. Ann NY Acad Sci 163:848–859, 1969.
82. SPATZ M, MCDANIEL EG, and LAQUEUR GL. Cycasin excretion in conventional and germ-free rats. Proc Soc Exp Biol Med 121:417–422, 1966.
83. KOBAYASHI A, and MATSUMOTO H. Biochemical studies on cycasin. III. Isolation of the aglycon, methylazoxymethanol, and its biological and chemical properties. Kagoshima Daigaku Nogakubu Gakujutsu Hokoku 16:1–10. 1966.
84. KOBAYASHI A, and MATSUMOTO H. Studies on methylazoxymethanol, the aglycone of cycasin (toxic constituent of *Cycas circinalis* nuts): isolation, biological and chemical properties. Arch Biochem Biophys 110:373–380, 1964.
85. HIRONI I, SHIBUYA C, and FUSHIMI K. Tumor induction in C57BL/6 mice by a single administration of cycasin. Cancer Res 29:1658–1662, 1969.
86. HIRONO I, and SHIBUYA C. High incidence of pulmonary tumors in dd mice by a single injection of cycasin (beta-D-glucosyloxyazoxymethane). Gann 61:403–407, 1970.
87. HIRONO I, HAYASHI K, MORI H, ET AL. Carcinogenic effects of cycasin (beta-D-glucosyloxyazoxymethane) in Syrian golden hamsters and the transplantability of induced tumors. Cancer Res 31:283–287, 1971.
88. HIRONO I, LAQUEUR GL, and SPATZ M. Tumor induction in Fischer and Osborne-Mendel rats by a single administration of cycasin. J Natl Cancer Inst 40:1003–1010, 1968.
89. SPATZ M, and BEALE M. Hydrolysis of cycasin by beta-D-glucosidase in skin of newborn rats. Proc Soc Exp Biol Med 128:1005–1008, 1968.
90. LAQUEUR GL. Cancer and ingestion of cycad seeds. *In* Primary Hepatoma (Burdett WJ, ed). Salt Lake City, University of Utah Press, 1965, pp 113–120.
91. FUKUNISHI R, TERASHI S, WATANABE K, ET AL. High yield of hepatic tumors in rats by cycasin. Gann 63:575–578, 1972.
92. KAWAJI K, FUKUNISHI R, TERASHI SI, ET AL. Induction of mammary cancer in rats with cycasin: a preliminary report. Gann 59:361, 1968.
93. GUSEK W, BUSS H, and LAQUEUR GL. Histological-histochemical investigations on "interstitial cycasin-tumor" of rat kidneys. Beitr Pathol 135:53–74, 1967.
94. HIRONO I. Carcinogenicity and neurotoxicity of cycasin with special reference to species differences. Fed Proc 31:1493–1497, 1972.
95. JONES M, SWEELEY C, and YANG M. Lipid composition of the cerebellum and spinal cord in postnatal cycasin-treated Swiss albino mice: preliminary observations. Fed Proc 31:1512–1516, 1972.
96. HIRANO A, and JONES M. Fine structure of cycasin-induced cerebellar alterations. Fed Proc 31:1517–1519, 1972.
97. KURLAND LT. An appraisal of the neurotoxicity of cycad and the etiology of amyotropic lateral sclerosis on Guam. Fed Proc 31:1540–1542, 1972.
98. SANGER VL, YANG M, and MICKELSEN O. Cycasin-induced central nervous system lesions in postnatal mice. Fed Proc 31:1524–1528, 1972.
99. JONES M, YANG M, and MICKELSEN O. Effects of methylazoxymethanol glucoside and methylazoxymethanol acetate on the cerebellum of the postnatal Swiss albino mouse. Fed Proc 31:1508–1511, 1972.
100. DASTUR DK. Cycad toxicity in monkeys: clinical, pathological and biochemical aspects. Fed Proc 23:1368–1369, 1964.
101. LAQUEUR GL, and SPATZ M. Toxicology of cycasin. Cancer Res 28:2262–2267, 1968.
102. HIRONO I, and SHIBUYA C. Induction of a neurological disorder by cycasin in mice. Nature 216:1311–1312, 1967.
103. HIRONO I, SHIBUYA C, and HAYASHI K. Induction of a cerebellar disorder with cycasin in newborn mice and hamsters. Proc Soc Exp Biol Med 131:593–599, 1969.
104. NARISAWA T, and NAKANO H. Carcinoma of the large intestine of rats induced by rectal infusion of methylazoxymethanol. Gann 64:93–95, 1973.
105. ZEDECK MS, and STERNBERG SS. A model system for studies of colon carcinogenesis: tumor induction by a single injection of methylazoxymethanol acetate. J Natl Cancer Inst 53:1419–1421, 1974.
106. ZEDECK MS, STERNBERG SS, YATAGANAS X, ET AL. Early changes induced in rat liver by methylazoxymethanol acetate: mitotic abnormalities and polyploidy. J Natl Cancer Inst 53:719–724, 1974.
107. RABE A, and HADDAD RK. Methylazoxymethanol-induced microencephaly in rats. Behavioral studies. Fed Proc 31:1536–1539, 1972.
108. ARIKAWA T. Antitumor activity of cycasin, a toxic glycoside of *Cycas revoluta* on Ehrlich ascites carcinoma and uterine cancer. Igaku Kenkyu 31:833–877, 1961.
109. EISEN MJ. Betel chewing among natives of the southwest Pacific Islands. Cancer Res 6:139–141, 1946.
110. ORR IM. Oral cancer in betel nut chewers in Travancore. Its etiology, pathology and treatment. Lancet 2:575–580, 1933.
111. FRIEDELL HL, and ROSENTHAL LM. The etiologic role of chewing tobacco in cancer of the mouth. Report of 8 cases treated with radiation. JAMA 116:2130–2135, 1941.
112. MAXWELL JL. Betel-chewing and cancer. Br J Cancer 1:729, 1924.
113. WELLS CR. Betel nut chewing and its effects. US Naval Med Bull 22:437–439, 1925.
114. ELLIS AG. Betel nut chewing and its effects. Including cancer of the mouth. Arch Intern Med 28:252–267, 1921.
115. MENDELSON RW, and ELLIS AG. Cancer as a public health problem in Siam. J Trop Med Hyg 27:274–278, 1924.

116. FELLS A. Cancer of the mouth in southern India, with an analysis of 209 operations. Br Med J 1:1357–1358, 1908.
117. BENTALL WG. Cancer in Travancore, South India. A summary of 1,700 cases. Br Med J 2:1428–1431, 1908.
118. DAVIDSON J. Betel chewing and cancer. Br Med J 2:733–734, 1923.
119. SPITTEL RL. Betel-chewing and cancer. Br Med J 1:158, 1924.
120. MARSDEN ATH. Betel cancer in Malaya. Med J Malaysia 14:162–165, 1960.
121. BALENDRA W. Symposium on oral diseases in tropical countries. I. The effect of betel chewing on the dental and oral tissues and its possible relationship to buccal carcinoma. Br Dent J 87:83–87, 1949.
122. ATKINSON L, CHESTER IC, SMYTH FG, ET AL. Oral cancer in New Guinea. Cancer 17:1289–1298, 1964.
123. MUIR CS, and KIRK R. Betel, tobacco and cancer of the mouth. Br J Cancer 14:597–608, 1960.
124. DAVIS GG. Buyo cheek cancer with special reference to etiology. JAMA 64:711–718, 1915.
125. WOELFEL WC, SPIES IW, and CLINE JK. Cancer of the mouth. I. Some chemical aspects of Buyo-cheek cancer. Cancer Res 1:748–749, 1941.
126. DUNHAM LJ, and HERROLD KM. Failure to produce tumors in the hamster cheek pouch by exposure to ingredients of betel quid; histopathologic changes in the pouch and other organs by exposure to known carcinogens. J Natl Cancer Inst 29:1047–1067, 1962.
127. SURI K, GOLDMAN HM, and WELLS H. Carcinogenic effect of a dimethyl sulphoxide extract of betel nut on the mucosa of the hamster buccal pouch. Nature 230:383–384, 1971.
128. ROWNTREE LG, LANSBURY J, and STEINBERG A. Neoplasms in albino rats resulting from the feeding of crude wheat germ oil made by ether-extraction. Proc Soc Exp Biol Med 36:424–426, 1937.
129. DORRANCE GM, and CICCONE EF. Production of sarcoma in albino rats as a result of feeding crude wheat germ oil. Proc Soc Exp Biol Med 36:426–427, 1937.
130. ROWNTREE LG, STEINBERG A, DORRANCE GM, ET AL. Sarcoma in rats from the ingestion of a crude wheat germ oil made by ether extraction. Am J Cancer 31:359–372, 1973.
131. ROWNTREE LG, STEINBERG A, DORRANCE GM, ET AL. Sarcoma in rats resulting from the ingestion of wheat germ oil made by ether extraction. Penn Med J 41:784–788, 1938.
132. CARRUTHERS C. Attempt to produce sarcomas in rats from ingestion of crude wheat germ oil by ether extraction. Proc Soc Exp Biol Med 40:107–108, 1939.
133. HALTER CR. Wheat germ oil and tumor formation. Proc Soc Exp Biol Med 40:257 258, 1939.
134. EVANS HM, and EMERSON GA. Failure to produce abdominal neoplasms in rats receiving wheat germ oil extracted in various ways. Proc Soc Exp Biol Med 41:318–320, 1939.
135. DINGEMANSE E, and VAN ECK WF. Wheat germ oil and tumor formation. Proc Soc Exp Biol Med 41:622–624, 1939.
136. AUCHINCLOSS R, and HAAGENSEN CD. Failure of wheat germ oil to produce neoplasms. Proc Soc Exp Biol Med 42:703–704, 1939.
137. GINZTON LL, and CONNOR CL. Crude wheat germ as a factor of tumor formation in rats. Am J Cancer 38:90–91, 1940.
138. BLUMBERG H. Attempts to produce tumors in rats by feeding crude wheat germ oil made by prolonged ether extraction. Public Health Rep 55:531–537, 1940.
139. BRUES AM, MARBLE BB, and RIEGEL B. Failure to induce sarcoma in rats with wheat germ oil preparations. Cancer Res 1:815–817, 1941.
140. RIDER DL. An experiment in feeding wheat germ oil prepared by ether extraction. Am J Cancer 38:275–276, 1946.
141. HARRIS PN. On the production of sarcoma with wheat germ oil. Cancer Res 7:26–34, 1947.
142. ROWNTREE LG, and ZIEGLER WM. Further report on malignancy observed in rats injected with crude ether-extracted wheat germ oil. Proc Soc Exp Biol Med 54:121–123, 1943.
143. DAY HG, BECKER JE, and MCCOLLUM EV. Effect of ether peroxides in wheat germ oil on production of tumors in rats. Proc Soc Exp Biol Med 40:21–22, 1939.
144. DUNHAM LG. A geographic study of the relationships between oral cancer and plants. Cancer Res 28:2369–2371, 1968.
145. O'GARA R. Phytogeographic relationships in cancer of the alimentary tract. *In* Carcinoma of the Alimentary Tract: Etiology and Pathogenesis (Burdette WJ, ed). Salt Lake City, University of Utah Press, 1965, 165–172.
146. MORTON JF. Plants associated with esophageal cancer cases in Curacao. Cancer Res 28:2268–2271, 1968.
147. MORTON JF. Tentative correlations of plant usage and esophageal cancer zones. Econ Bot 24:217–226, 1970.
148. MORTON JF. A survey of medicinal plants of Curacao. Econ Bot 22:87–102, 1968.
149. DOLL R. The geographical distribution of cancer. Br J Cancer 23:1–8, 1969.
150. DUNHAM LJ, and BAILAR J. World maps of cancer mortality rates and frequency ratios. J Natl Cancer Inst 41:155–203, 1968.
151. CHAKLIN AV. Geographical differences in the distribution of malignant tumours. Bull WHO 27:337–358, 1962.
152. MASON M, BAILAR J, and EISENBERG H. Geographical variation in the incidence of esophageal cancer. J Chronic Dis 17:667–676, 1964.
153. DAVIDSON EC. Tannic acid in the treatment of burns. Surg Gynecol Obstet 41:202, 1925.
154. ERB IH, MORGAN EM, and FARMER AW. The pathology of burns. Ann Surg 117:234–255, 1948.
155. WELLS DB, HUMPHREY HD, and COLL JJ. The relation of tannic acid to the liver necrosis occurring in burns. N Engl J Med 226:629–635, 1942.
156. BAKER RD, and HANDLER P. Animal experiments with tannic acid. Ann Surg 118:417–426, 1943.
157. CAMERON GR, MILTON RF, and ALLEN JW. Toxicity of tannic acid. An experimental investigation. Lancet 2:179–186, 1943.
158. KORPASSY B, and KOVACS K. Experimental liver cirrhosis in rats produced by prolonged subcutaneous administration of tannic acid. Br J Exp Pathol 30:266–272, 1943.
159. FORBES JC, and EVANS EI. Tannic acid and liver necrosis. Surg Gynecol Obstet 76:612–613, 1943.
160. KORPÁSSY B, and KOVACS K. Haemorrhagic gastric erosions and duodenum pigmentation in rats following parenteral administration of tannic acid. Acta Physiol Acad Sci Hung 1:125–130, 1950.
161. KORPÁSSY B. Hepatomes et cholangiomes provoques chez le rat par administration sous-cutanee d'acide tannique. Bull Cancer 37:52–56, 1950.
162. KORPÁSSY B, and MOSONYI M. The carcinogenic activity of tannic acid. Liver tumours induced in rats by prolonged subcutaneous administration of tannic acid solutions. Br J Cancer 4:411–420, 1950.
163. KORPÁSSY B, and MOSONYI M. The carcinogenic action of tannic acid. Effect of casein on the development of liver tumours. Acta Morphol Acad Sci Hung 1:37–54, 1951.
164. KORPÁSSY B, and MOSONYI M. Influence of dietetic factors on carcinogenic activity of tannic acid. Lancet 1:1416, 1951.
165. KORPÁSSY B, and MOSONYI M. Influence of dietary protein on the carcinogenic activity of tannic acid. Acta Morphol Acad Sci Hung 3:353–362, 1953.
166. KORPÁSSY B. The hepatocarcinogenicity of tannic acid. Cancer Res 19:501–504, 1959.
167. KIRBY KS. Induction of tumours by tannin extracts. Br J Cancer 14:147–150, 1960.

168. MOSONYI M, and KORPÁSSY B. Rapid production of malignant hepatomas by simultaneous administration of tannic acid and 2-acetylaminofluorene. Nature 171:791, 1953.
169. O'GARA RW. Biological screening of selected plant material for carcinogens. Cancer Res 28:2272–2275, 1968.
170. O'GARA RW, LEE C, and MORTON JF. Carcinogenicity of extracts of selected plants from Curacao after oral and subcutaneous administration to rodents. J Natl Cancer Inst 46:1131–1137, 1971.
171. O'GARA RW, LEE CW, MORTON JF, ET AL. Sarcoma induced in rats by extracts of plants and by fractionated extracts of *Krameria ixina*. J Natl Cancer Inst 52:445–448, 1974.
172. DUNHAM LJ, SHEETS RH, and MORTON JF. Proliferative lesions in cheek pouch and esophagus of hamsters treated with plants from Curacao, Netherland Antilles. J Natl Cancer Inst 53:1259–1270, 1974.
173. PRADHAM SN, CHUNG EB, GHOSH B, ET AL. Potential carcinogens. I. Carcinogenicity of some plant extracts and their tannin-containing fractions in rats. J Natl Cancer Inst 52:1579–1582, 1974.
174. MAGEE PN, and BARNES JM. Carcinogenic nitroso compounds. Adv Cancer Res 10:163–246, 1967.
175. DRUCKREY H, PREUSSMANN R, IVANKOVIC S, ET AL. Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitroso-Verbindungen an BD-Ratten. Z Krebsforsch 69:103–201, 1967.
176. CHALLIS BC, and BARTLETT CD. Possible cocarcinogenic effects of coffee constituents. Nature 254:532–533, 1975.
177. SEGI M. Tea-gruel as a possible factor for cancer of the esophagus. Gann 66:199–202, 1975.
178. KAISER HE, and BARTONE JC. The carcinogenic activity of ordinary tea. A preliminary note. J Natl Med Assoc 58:361, 1966.
179. KAISER HE. Cancer-promoting effects of phenols in tea. Cancer 20:614–616, 1967.
180. FONG HHS, BHATTI W, and FARNSWORTH NR. Antitumor activity of certain plants due to tannins. J Pharm Sci 61:1818, 1972.
181. WALL ME, TAYLOR H, AMBROSIO L, ET AL. Plant antitumor agents. III. A convenient separation of tannins from other plant constituents. J Pharm Sci 58:839–841, 1969.
182. LOUB WD, FONG HHS, THEINER M, ET AL. Partial characterization of antitumor tannin isolated from *Calycogonium squamulosum* (Melastomataceae). J Pharm Sci 62:149, 1973.
183. HARTWELL JL, and ABBOTT BJ. Antineoplastic principles in plants: recent developments in the field. Adv Pharmacol Chemother 7:117–209, 1969.
184. ERUSALIMSKII AL, and KAZMIN SD. The biological and chemical basis for the possible use of tanning substances for the treatment of patients with malignant tumors. Vrach Delo 8:58–61, 1965.
185. LASNITSKI A. Note on the action of tannin upon tumor glycolysis. Biochem J 40:263–264, 1946.
186. SCHOENTAL R. Toxicology and carcinogenic action of pyrrolizidine alkaloids. Cancer Res 28:2237–2246, 1968.
187. MCLEAN EK. The toxic action of pyrrolizidine (*Senecio*) alkaloids. Pharmacol Rev 22:429–483, 1970.
188. BULL LB, CULVENOR CCJ, and DICK AT. The pyrrolizidine alkaloids. New York, John Wiley, 1968.
189. DYBING O, and ERICHSEN S. Liver changes in rats after administration of *Senecio aquaticus*. Acta Pathol Microbiol Scand [A] 47:1–8, 1959.
190. HARRIS PN, and CHEN KK. Development of hepatic tumors in rats following ingestion of *Senecio longilobus*. Cancer Res 30:2881–2886, 1970.
191. SCHOENTAL R, and CAVANAUGH JB. Brain and spinal cord tumors in rats treated with pyrrolizidine alkaloids. J Natl Cancer Inst 49:665–669, 1972.
192. SCHOENTAL R, FOWLER ME, and COADY A. Islet cell tumors of the pancreas found in rats given pyrrolizidine alkaloids from *Amsinckia intermedia* Fisch and Mey and from *Heliotropium supinum* L. Cancer Res 30:2127–2131, 1970.
193. SVOBODA DJ, and REDDY JK. Lasiocarpine-induced, transplantable squamous cell carcinoma of the rat skin. J Natl Cancer Inst 53:1415–1418, 1974.
194. ALLEN JR, HSU IC, and CARSTENS LA. Dehydroretonecine-induced rhabdomyosarcomas in rats. Cancer Res 35:997–1002, 1975.
195. CULVENOR CCJ. Tumor-inhibitory activity of pyrrolizidine alkaloids. J Pharm Sci 57:1112–1117, 1968.
196. PUKHALSKAYA ECH, PETROVA MF, and MANKO IW. The effect of six alkaloids (1-methylpyrrolizidine derivatives) upon the growth of hepatomas and several other mutually inoculated animal tumors. Byull Eksper Biol Med 48:91–93, 1959.
197. KUPCHAN SM, DOSKOTCH RW, and VANEVENHOVEN PW. Tumor inhibitors. III. Monocrotaline, the active principle of *Crotalaria spectabilis*. J Pharm Sci 53:343–345, 1964.
198. SCHOENTAL R, HARD GC, and GIBBARD S. Histopathology of renal lipomatous tumors in rats treated with the "natural" products, pyrrolizidine alkaloids and α,β-unsaturated aldehydes. J Natl Cancer Inst 47:1037–1044, 1971.
199. SCHOENTAL R, HEAD MA, and PEACOCK PR. *Senecio* alkaloids. Primary liver tumours in rats as a result of treatment with (1) a mixture of alkaloids from *S. jacobaea* Linn.; (2) retrorsine; (3) isatidine. Br J Cancer 8:458–465, 1954.
200. COOK J, DUFFY E, and SCHOENTAL R. Primary liver tumours in rats following feeding with alkaloids of *Senecio jacobaea*. Br J Cancer 4:405–410, 1950.
201. CAMPBELL JG. An investigation of the hepatotoxic effects in the fowl of ragwort (*Senecio jacobaea* Linn) with special reference to the induction of liver tumours with seneciphylline. Proc R Soc Edinb [Biol] B 66:111–130, 1956.
202. SCHOENTAL R, and HEAD MA. Pathological changes in rats as a result of treatment with monocrotaline. Br J Cancer 9:229–237, 1955.
203. SCHOENTAL R, and BENSTED JPM. Effects of whole body irradiation and of partial hepatectomy on the liver lesions induced in rats by a single dose of retrorsine, a pyrrolizidine (*Senecio*) alkaloid. Br J Cancer 17:242–251, 1963.
204. SCHOENTAL R, and HEAD MA. Progression of liver lesions produced in rats by temporary treatment with pyrrolizidine (*Senecio*) alkaloids. Br J Cancer 11:535–544, 1957.
205. SVOBODA DJ, and REDDY JK. Malignant tumors in rats given lasiocarpine. Cancer Res 32:908–911, 1972.
206. HAKIM SAE. Death, cardio-myopathy, symptomless glaucoma and cancer from edible oils containing *Argemone*. Maharashtra Med J 17:109–130, 1970.
207. SARKAR SN. Isolation from *Argemone* oil of dihydrosanguinarine and sanguinarine. Toxicity of sanguinarine. Nature 162:265–266, 1948.
208. HAKIM SAE, MIJOVIC V, and WALKER J. Experimental transmission of sanguinarine in milk: detection of a metabolic product. Nature 189:201–204, 1961.
209. SHAMMA M. The Isoquinoline Alkaloids; Chemistry and Pharmacology. New York, Academic Press, 1972, p 341.
210. MESSMER WM, TIN-WA M, FONG HHS, ET AL. Fagaronine, a new tumor inhibitor isolated from *Fagara zanthoxyloides* Lam. (Rutaceae). J Pharm Sci 61:1858–1859, 1972.
211. TIN-WA M, BELL CL, BEVELLE C, ET AL. Potential anticancer agents. I. Confirming evidence for the structure of fagaronine. J Pharm Sci 63:1476–1477, 1974.
212. DU PLESSIS LS, NUNN JR, and ROACH WA. Carcinogen in a Transkeian Bantu food additive. Nature 222:1198–1199, 1969.
213. LONG EL, NELSON AA, FITZHUGH OG, ET AL. Liver tumors produced in rats by feeding safrole. Arch Pathol 75:595–604, 1963.

214. LONG EL, HANSEN WH, and NELSON AA. Liver tumors produced in rats by feeding safrole. Fed Proc 20:287, 1961.
215. HOMBURGER F, FRIEDLER G, KELLEY T, JR, ET AL. 4-Allyl-1,2-methylenedioxybenzene (safrole) as a new tool for the study of pathogenesis of toxic hepatic cirrhosis and adenomatosis. Fed Proc 20:288, 1961.
216. HOMBURGER F, KELLEY T, JR, FRIEDLER G, ET AL. Toxic and possible carcinogenic effects of 4-allyl-1,2-methylenedioxybenzene (safrole) in rats on deficient diets. Med Exp 4:1–11, 1961.
217. HOMBURGER F, KELLEY T, JR, BAKER TR, ET AL. Sex effect on hepatic pathology from deficient diet and safrole in rats. Arch Pathol 73:118–125, 1962.
218. HOMBURGER F, KELLEY T, JR, and FRIEDLER G. Nutritional factors modifying hepatic adenomatosis induced by safrole (4-allyl-1,2-methylenedioxybenzene). Proc Am Assoc Cancer Res 3:236, 1961.
219. HOMBURGER F, KELLEY TF, and BOGDONOFF PD. Effects of diet on toxicity of safrole in rats. Toxicol Appl Pharmacol 7:485, 1965.
220. HOMBURGER F, BOGDONOFF PD, and KELLEY TF. Influence of diet on chronic oral toxicity of safrole and butter yellow in rats. Proc Soc Exp Biol Med 119:1106–1110, 1965.
221. HAGAN EC, JENNER PM, JONES WI, ET AL. Toxic properties of compounds related to safrole. Toxicol Appl Pharmacol 7:18–24, 1965.
222. STONER GD, SHIMKIN MB, KNIAZEFF AJ, ET AL. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res 33:3069–3085, 1973.
223. ABBOTT DD, PACKMAN EW, WAGNER EM, ET AL. Chronic oral toxicity of oil of sassafras and safrole. Pharmacologist 3:62, 1961.
224. TAYLOR JM, JENNER PM, and JONES WI. A comparison of the toxicity of some allyl, propenyl and propyl compounds in the rat. Toxicol Appl Pharmacol 6:378–387, 1964.
225. INNES JRM, ULLAND BM, VALERO MG, ET AL. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J Natl Cancer Inst 42:1101–1114, 1969.
226. EPSTEIN SS, FUJII K, ANDREA J, ET AL. Carcinogenicity testing of selected food additives by parenteral administration in infant mice. Toxicol Appl Pharmacol 16:321–334, 1970.
227. TAYLOR JM, and JONES WI. Metabolism of safrole in the rat. Fed Proc 20:432, 1961.
228. STILLWELL WG, CARMAN MJ, BELL L, ET AL. The metabolism of safrole and 2′,3′-epoxysafrole in the rat and guinea pig. Drug Metab Dispos 2:489–498, 1974.
229. BORCHERT P, MILLER JA, MILLER EC, ET AL. 1′-Hydroxysafrole, a proximate carcinogenic metabolite of safrole in the rat and mouse. Cancer Res 33:590–600, 1973.
230. BORCHERT P, WISLOCKI PG, MILLER JA, ET AL. The metabolism of the naturally occurring hepatocarcinogen safrole to 1-hydroxysafrole and the electrophilic reactivity of 1-acetoxysafrole. Cancer Res 33:575–589, 1973.
231. FISHBEIN L, FAWKES J, FALK HL, ET AL. Thin-layer chromatography of the rat bile and urine following intravenous administration of safrole, isosafrole and dihydrosafrole. J Chromatogr 29:267–273, 1967.
232. OSWALD EO, FISHBEIN L, CORBETT BJ, ET AL. Identification of tertiary aminomethylenedioxypropiophenones as urinary metabolites of safrole in the rat and guinea pig. Biochim Biophys Acta 230:237–247, 1971.
233. OSWALD EO, FISHBEIN L, CORBETT BJ, ET AL. Urinary excretion of tertiary aminomethylenedioxypropiophenones as metabolites of myristicin in the rat and guinea pig. Biochim Biophys Acta 244:322–328, 1971.
234. WISLOCKI PG, BORCHERT P, MILLER EC, ET AL. 1′-Hydroxysafrole: a proximate carcinogenic metabolite of safrole. Proc Am Assoc Cancer Res 13:12, 1972.
235. BORCHERT P, MILLER JA, MILLER EC, ET AL. 1′-Hydroxysafrole, a new metabolite, and 1′-acetoxy safrole, a reactive derivative of safrole. Proc Am Assoc Cancer Res 12:34, 1971.
236. BORCHERT P. 1′-Hydroxysafrole: a proximate carcinogenic metabolite of safrole in the rat and mouse. PhD thesis, Madison, University of Wisconsin, 1972.
237. LONG EL, and JENNER PM. Esophageal tumors produced in rats by the feeding of dihydrosafrole. Fed Proc 22:275, 1963.
238. GOSALVEZ M, and MERCHAN J. Induction of rat mammary adenomas with the respiratory inhibitory rotenone. Cancer Res 33:3047–3050, 1973.
239. LAWRENCE JC. The effect of asiaticoside on guinea pig skin. J Invest Dermatol 49:95–96, 1967.
240. LAWRENCE JC. The morphological and pharmacological effects of asiaticoside upon skin in vitro and in vivo. Eur J Pharmacol 1:414–424, 1967.
241. LAERUM OD, and IVERSEN OH. Reticuloses and epidermal tumors in hairless mice after topical skin applications of cantharidin and asiaticoside. Cancer Res 32:1463–1469, 1972.
242. LEDINKO N, and EVANS M. Enhancement of adenovirus transformation of hamster cells by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine, caffeine and hydroxylamine. Cancer Res 33:2936–2938, 1973.
243. DONOVAN PJ, and DIPAOLO JA. Caffeine enhancement of chemical carcinogen-induced transformation of cultured Syrian hamster cells. Cancer Res 34:2720–2727, 1974.
244. WAGENER DJT. Enhanced growth of fibrosarcoma in mice treated with phytohemagglutinin. Cancer Res 33:1295–1300, 1973.
245. HARTWELL JL. Survey of compounds which have been tested for carcinogenic activity (Public Health Service Publication No. 149). Washington, DC, US Government Printing Office, 1951.
246. SHUBIK P, and HARTWELL JL. Survey of compounds which have been tested for carcinogenic activity (Public Health Service Publication No. 149, supplement 1). Washington, DC, US Government Printing Office, 1957.
247. SHUBIK P, and HARTWELL JL. Survey of compounds which have been tested for carcinogenic activity (Public Health Service Publication No. 149, supplement 2). Washington, DC, US Government Printing Office, 1969.
248. Survey of compounds which have been tested for carcinogenic activity (Department of Health, Education, and Welfare Publication No. [NIH] 73–453; Public Health Service Publication No. 149). Washington, DC, US Government Printing Office, 1970–1971.
249. CASARETT LJ, and DOULL J. Toxicology, the Basic Science of Poisons. New York, MacMillan Publishing Co, 1975.
250. TYIHAK E. Chemical studies on the plant "antibiotics." Herba Pol 16:321–328, 1970 (*abstracted in* Chem Abstr 75:59792m, 1970).
251. HIRONO I, SHIMIZU M, FUSHIMI K, ET AL. Carcinogenic activity of *Petasites japonicus* Maxim., a kind of coltsfoot. Gann 64:527–528, 1973.
252. KIER LB, FITZGERALD DB, and BURGETT S. Isolation of podophyllotoxin from *Callitris drummondi*. J Pharm Sci 52:502–503, 1963.
253. SEREBRYAKOVA AP, FILITIS LN, and UTKIN LM. Lignans of the Soviet Union *Juniperus* plants. Zh Obshch Khim 31:1731–1734, 1961.
254. HARTWELL JL, JOHNSON JM, FITZGERALD DB, ET AL. Podophyllotoxin from *Juniperus* species: savinin. J Am Chem Soc 75:235–236, 1953.
255. FARNSWORTH NR. Pflanzen als Quelle neuer Arzneimittel gegen Krebs. Pharm-Ztg 113:1293–1307, 1968.
256. KUPCHAN SM, HEMMINGWAY JC, and KNOX JR. Tumor

inhibitors. VII. Podophyllotoxin, the active principle of *Juniperus virginiana*. J Pharm Sci 55:659–660, 1965.
257. RIGGS NV. The occurrence of macrozamin in the seeds of cycads. Aust J Chem 7:123–124, 1954.
258. PALEKAR RS, and DASTUR DK. Cycasin content of *Cycas circinalis*. Nature 206:1363–1365, 1965.
259. WILLIAMS JN, JR, and LAQUEUR GL. Response of liver nucleic acids and lipids in rats fed *Cycas circinalis* endosperm or cycasin. Proc Soc Exp Biol Med 118:1–4, 1965.
260. NAGAHAMA T, IJUIN I, and WATABA T. Azoxyglycosides occurring in the outer shells of *Cycas circinalis* nuts. Agric Biol Chem 28:575–576, 1964.
261. DASTUR DK, and PALEKAR RS. Effect of boiling and storing on cycasin content of *Cycas circinalis*. Nature 210:841–843, 1966.
262. KOBAYASHI A, and MUROZONO T. Large-scale preparation of cycasin. Kagoshima Daigaku Nogakubu Gakujutsu Hokoku 21:129–843, 1966.
263. NISHIDA K, KOBAYASHI A, and NAGAHAMA T. Cycasin, a toxic glycoside of *Cycas revoluta*. V. Quantitative determination of cycasin in cycad seeds. Bull Agric Chem Soc Jpn 20:74–76, 1956.
264. NUMATA T, NAGAHAMA T, and NISHIDA K. Some new azoxyglycosides of *Cycas revoluta*. IV. Azoxyglycosides found in the male strobil. Mem Fac Agric Kagoshima Univ 4:5–8, 1960.
265. DOSSAJI SF, and HERBIN GA. Occurrence of macrozamin in the seeds of *Encephalartos hildebrandtii*. Fed Proc 31:1470–1472, 1972.
266. ALTENKIRK B. Occurrence of macrozamin in the seeds of *Encephalartos transvenosus* and *E. lanatus*. Lloydia 37:636–637, 1974.
267. RIGGS NV. Nature of a cyclitol isolated from *Macrozamia riedlei*. J Chem Soc, 1949, pp 3199–3200.
268. DELONG V, HAVRLIKOVA J, and SANTAVY F. Isolation and identification of the alkaloids in some rare *Colchicum* species. II. Ann Pharm Fr 13:449–454, 1955.
269. FELL KR, and RAMSDEN D. *Colchicum:* a review of colchicums and the sources, chemistry, biogenesis and assay of colchicine and its congeners. Lloydia 30:123–140, 1967.
270. WILDMAN WC. Colchicine and related compounds. *In* Alkaloids (Manske RHF, ed). New York, Academic Press, 1960, vol 6, pp 247–288.
271. WILDMAN WC, and PURSEY BA. Colchicine and related compounds. *In* Alkaloids (Manske RHF, ed). New York, Academic Press, 1968, vol 11, pp 407–457.
272. COOK JW, and LOUDON JD. Colchicine. *In* Alkaloids (Manske RHF, and Holmes HL, eds). New York, Academic Press, 1952, vol 2, pp 261–329.
273. WILLAMAN JJ, and SCHUBERT BG. Alkaloid-bearing plants and their contained alkaloids (Technical Bulletin No. 1234). Washington, DC, Agricultural Research Service, US Department of Agriculture, 1961.
274. KAUL JL, MOZA BK, SANTAVY F, ET AL. Substances from the plants of the subfamily Wurmbaeoideae and their derivatives. LIX. Isolation of alkaloids from some plants of the subfamily Wurmbaeoideae. Coll Czech Chem Commun 29:1689–1701, 1964.
275. SANTAVY F, and COUFALIK E. Substances of meadow saffron and their derivatives. XXIV. Isolation of substances from the corms of various species of the family Liliaceae. Coll Czech Chem Commun 16:198–203, 1951.
276. DELONG V, HAVRLIKOVA J, and SANTAVY F. Isolation and identification of the alkaloids in some rare *Colchicum* species. II. Ann Pharm Fr 13:449–454, 1955.
277. AVRAMOVA B, and IVANOV V. The alkaloid content of uncultivated *Colchicum autumnale*, in Bulgaria. I. A study of the seeds. Tr Nauchn Inst Farm 3:7.5–81, 1961.
278. AVRAMOVA B, and IVANOV V. The alkaloid content of uncultivated *Colchicum autumnale*, in Bulgaria. II. Studies on other parts of the plant. Tr Nauchn Inst Farm 3:81–86, 1961.
279. HILL RD, and UNRAU AM. Biosynthesis of colchicine. Can J Chem 43:709–711, 1965.
280. AVRAMOVA B, and IVANOV V. The alkaloid content of uncultivated *Colchicum autumnale*, in Bulgaria. II. Studies on other parts of the plant. Tr Nauchn Inst Farm 3:81–86, 1961.
281. POTESILOVA H, ALCARAZ C, and SANTAVY F. Substances from the plants of the subfamily Wurmbaeoideae and their derivatives. LXII. Isolation of alkaloids from the plants *Colchicum byzanthinum, C. cupani, C. libanoticum, Merendera filifolia, C. luteum,* and *Kreysigia multiflora*. Coll Czech Chem Commun 34:2128–2133, 1969.
282. HAMIDI A, and FAHMY MA. An investigation of *Colchicum cornigerum*. J Chem UAR 3:279, 1960.
283. EL-HAMIDI A, and SANTAVY F. Substances from the plants of the subfamily Melanthoid and their derivatives. LIII. Chemical investigation of *Colchicum cornigerum*. Coll Czech Chem Commun 27:2111–2118, 1962.
284. SANTAVY F, ZAJICEK DV, and NEMECKOVA A. Substances of *Colchicum autumnale* and their derivatives. XLVII. Isolation and identification of compounds from some plants related to *Colchicum autumnale*. Chem Listy 51:597–602, 1957.
285. MICEVSKA M, and PODOLESOV B. Determination of colchicine in seeds of the Macedonian strains of *Colchicum*. Arh Farm 18:31–33, 1968.
286. PODOLESOV B, and MICEVSKA M. Separation of colchicine from *Colchicum doerfleri* seeds. Arh Farm 20:183–185, 1970.
287. YUSUPOV MK, and SADYKOV AS. New alkaloids from *Colchicum kesselringii*. Zh Obshch Khim 34:1672–1676, 1964.
288. YUSUPOV MK, and SADYKOV AS. Alkaloids from *Colchicum kesselringii* seeds. Dokl Akad Nauk Uzb SSR 24:25–27, 1967.
289. YUSUPOV MK, and SADYKOV AS. Alkaloids of *Colchicum kesselringii* corms. Rast Resur 6:104–107, 1970.
290. WAHID MA, and SAMIULLAH. Standardization of active principles of indigenous pharmacopoeial drugs found in West Pakistan. Pakistan J Sci Ind Res 3:228–230, 1960 (abstracted in Chem Abstr 58:1301d, 1963).
291. SADYKOV AS, and YUSUPOV MK. Preparation of colchicine. USSR patent No. 166,459 (Cl. A 61k), Nov 19, 1964, applied Apr 7, 1962.
292. SADYKOV AS, YUSUPOV MK, and CHOMMADOV B. Composition of alkaloids in *Colchicum luteum* at different growth stages. Rast Resur 5:441–444, 1969.
293. PODOLESOV BD. Isolation of colchicine from *Colchicum macedonicum* seeds. Glas Hem Dr 28:461–463, 1963.
294. MICEVSKA M, and PODOLESOV B. Determination of colchicine in tubers of the Macedonian varieties of the genus *Colchicum*. Arh Farm 19:19–20, 1969.
295. MADINOVA V, and SANTAVY F. Substances of *Colchicum autumnale* and their derivatives. XXXVII. Compounds from the flowers and corms of *Colchicum speciosum*. Coll Czech Chem Commun 19:1283–1288, 1954.
296. KISELEV. Colchicine alkaloids in plant material. Khim Farm 2:43–46, 1968.
297. SANTAVY F, and BARTEK J. Isolation of substances from the tubers of *Gloriosa superba, G. rothschildiana,* and *G. simplex*. Pharmazie 7:595–598, 1952.
298. SANTAVY F, KINCL FA, and SHINDE AR. Isolation of principles of *Gloriosa superba*. Arch Pharm 290:376–385, 1957.
299. SANTAVY F. Presence of colchicine alkaloids in *Kreysigia multiflora*. Experientia 23:273, 1967.
300. TROZYAN AA, YUSUPOV MK, and SADYKOV AS. Merenderin, a new base from *Merendera raddeana*. Khim Prir Soedin 7:541–542, 1971.
301. KLEIN G, and POLLAUF G. Microchemical detection of alkaloids in plants. XII. The detection of colchicine. Oesterr Bot Z 78:251–256, 1930.

302. FESTER GA, RETAMAR JA, RICCIARDI AIA, ET AL. Essential oils from Argentina plants. XI. Rec Fac Ing Quim 28:9–17, 1959.
303. CROW WD, and GREET YM. The occurrence of reserpine in *Alstonia constricta*. Aust J Chem 8:460–463, 1955.
304. CURTIS RG, HANDLEY GJ, and SOMERS TC. Reserpine from *Alstonia constricta*. Chem Ind, 1955, pp 1598–1599.
305. DAS B, BIEMANN K, CHATTERJEE A, ET AL. Alkaloids of the bark of *Alstonia venenata* R. Br. Tetrahedron Lett, 1965, pp 2239–2244.
306. GOVINDACHARI TR, VISWANATHAN N, PAI BR, ET AL. Chemical constituents of *Alstonia venenata* R. Br. Tetrahedron 21:2951–2956, 1965.
307. DUTTA SC, and RAY AB. Root alkaloids of *Alstonia venenata*. Indian J Chem 13:98–100, 1975.
308. FARNSWORTH NR. The phytochemistry of *Vinca* species. *In* The *Vinca* Alkaloids, Botany, Chemistry and Pharmacology (Taylor WI, and Farnsworth NR, eds). New York, Marcel Dekker, 1973, pp 95–179.
309. TIN-WA M, and FARNSWORTH NR. The phytochemistry of minor *Catharanthus* species. *In* The *Catharanthus* Alkaloids (Taylor WI, and Farnsworth NR, eds). New York, Marcel Dekker, 1975, pp 85–124.
310. MACKO E, and RAFFAUF RF. Pharmacology of the alkaloids of *Excavatia coccinea*. J Med Pharm Chem 2:585–587, 1960.
311. DAY FA, and MOORE BP. Alkaloids of *Ochrosia poweri*. I. The leaf-bases. Aust J Chem 15:548–554, 1962.
312. KORZUN BP, ST ANDRE AF, and ULSHAFER PR. Paper chromatographic evaluation of *Rauwolfia* species. J Am Pharm Assoc 46:720–723, 1957.
313. BERNAL RM, VILLEGAS-CASTILLO A, and ESPEJO OP. Philippine species of *Rauwolfia*. I. Isolation of reserpine and rescinnamine from *Rauwolfia amsoniaefolia*. Experientia 16:353–355, 1960.
314. JACOBUCCI G, and DEULOFEU V. Alkaloids of *Rauwolfia schueli* and *Rauwolfia boliviana*. An Asoc Quim Org 46:143–148, 1958.
315. ROCHA DE MACEDO G. *Rauwolfia caffra*. Rev Port Farm 8:149–181, 1958.
316. KIDD DAA. Alkaloids of *Rauwolfia* species: preliminary studies on *Rauwolfia cambodiana*. Chem Ind, 1957, p 1013.
317. DILLEMANN G, and PARIS R. Two *Rauwolfia* of Laos. Compt Rend 244:1254–1256, 1957.
318. KOHLS MW, DRAPER MP, KELLER F, ET AL. The isolation of reserpine from *Rauwolfia canescens*. J Am Chem Soc 76:1381, 1954.
319. STOLL A, and HOFMANN A. Alkaloids from the leaves and roots of *Rauwolfia canescens*. Soc Biol Chemists, India, 1955, pp 248–258.
320. CHIANG HC, and CHEN WF. Determination of alkaloids in *Rauwolfia chinensis*. Chemistry, 1962, pp 95–97.
321. RAO DS, and RAO SB. The alkaloids of *Rauwolfia micrantha*. J Am Pharm Assoc 44:253–254, 1955.
322. COURT WE. The alkaloids of *Rauwolfia mombasiana* Stapf. Can J Pharm Sci 1:83–84, 1966.
323. SCHULER BOG, and WARREN FL. *Rauwolfia* alkaloids. I. Reserpine and ajmaline from *Rauwolfia natalensis*. J Chem Soc, 1956, pp 215–216.
324. TIMMINS P, and COURT WE. Root bark alkaloids of *Rauwolfia obscura*. Phytochemistry 13:1997, 1974.
325. TIMMINS P, and COURT WE. Root alkaloids of *Rauwolfia oreogiton*. Planta Med 26:170–173, 1974.
326. WAN ASC, and KIANG AK. Alkaloids of *Rauwolfia perakensis*. Proc Symp Phytochem Kuala Lumpur, 1957, pp 181–183.
327. KIANG AK, LEE H, and GOH J. Alkaloids in the leaves and stems of *Rauwolfia perakensis*. Lloydia 27:220, 1964.
328. HOCHSTEIN FA. Alkaloids of *Rauwolfia sellowii*. J Am Chem Soc 77:5744–5755, 1955.
329. PAKRASHI SC, DJERASSI C, WASICKY R, ET AL. Alkaloid studies. IX. *Rauwolfia* alkaloids. IV. Isolation of reserpine and other alkaloids from *Rauwolfia sellowii*. J Am Chem Soc 77:6687–6689, 1955.
330. MOREIRA EA. Identification of reserpine in the roots of *Rauwolfia sellowii*. Tr Farm 31:57–62, 1964.
331. MULLER JM, SCHLITTLER E, and BEIN HJ. Reserpine, the sedative compound from *Rauwolfia serpentina*. Experientia 8:338, 1952.
332. CHAUDHURY NA, and CHATTERJEE A. Alkaloids of *Rauwolfia sumatrana*. II. Isolation of ajmaline, delta-yohimbine, reserpine, and sarpagine. J Sci Ind Res 188:39B, 1959.
333. CARDOSO HT, and VENANCIO IAA. Identification of reserpine in *Rauwolfia ternifolia*. Rev Bras Biol 16:231–234, 1956.
334. DJERASSI C, and FISHMAN J. Alkaloid studies. VII. Tetraphyllin and tetraphyllicine, two new alkaloids from *Rauwolfia tetraphylla*. Chem Ind, 1955, pp 627–629.
335. DJERASSI C, FISHMAN J, GORMAN M, ET AL. Alkaloid studies. XVI. Alkaloids of *Rauwolfia tetraphylla* L. The structures of tetraphylline and tetraphyllicine. J Am Chem Soc 79:1217–1222, 1957.
336. VAN TAMELEN EE, and TAYLOR CW. The alkaloids of *Rauwolfia*. Structure of raunescine and isoraunescine. J Am Chem Soc 79:5256–5262, 1957.
337. POISSON J, LE HIR A, GOUTEREL R, ET AL. Isolation of reserpine from roots of *Rauwolfia vomitoria*. Compt Rend 238:1607–1609, 1954.
338. DUBOIS L. *Rauwolfia vomitoria*. Bull Agric Congo Beige 46:566–595, 1955.
339. FORBATH TP. Liquid extraction commercializes reserpine. Chem Eng 64:230–233, 1957.
340. WEI CH. The constituents of the roots of *Rauwolfia yunnanensis*. I. Isolation of ajmalicine and reserpine. Yao Hsueh Hsueh Pao 12:429–434, 1965.
341. HOLKER JSE, CAIS FA, HOCHSTEIN FA, ET AL. Alkaloid studies. XXII. Alkaloids of *Vallesia dichotoma*. J Org Chem 24:314–316, 1959.
342. OGNYANOV I, DALEV P, DUCHEVSKA KH B, ET AL. New alkaloids from *Vinca herbacea*. Riv Ital Essenze Perfumi Piante Offic Aromi Saponi Cosmet 47:600–602, 1960 (abstracted in Chem Abstr 65:5500a, 1966).
343. BORKOWSKI B, BATKIEWICZ E, and DROST K. Chromatographic search for reserpine in the herb *Vinca major*. Dissert Pharm 16:171–176, 1964.
344. LYAPUNOVA PM, and YU G. Investigation of *Vinca minor*. I. Alkaloidal constituents. Farm Zh 16:42–47, 1961.
345. LYAPUNOVA PN. A study of the alkaloid constituents of *Vinca minor* growing in the Ukraine. Izuch I Ispol'z Lekarstv Rast Resur SSSR Sb, 1964, pp 255–257.
346. THOMAS DW, and BIEMANN K. The alkaloids of *Voacanga africana*. Lloydia 31:1–8, 1968.
347. SAIKI Y, AKAHORI Y, NORO T, ET AL. Gas chromatography of natural volatile oils. II. Gas chromatography of the volatile oils of plants belonging to *Asiasarum* and *Asarum* genera. Yakagaku Zasshi 87:1529–1534, 1967.
348. KAKU LT, and KONDO T. Essential oil of valerian and burdock. Yakagaku Zasshi 51:8–17, 1931.
349. SAIKI Y, SAITO T, SASAKI H, ET AL. Gas-chromatography of natural volatile oils. I. Volatile oil of the Chinese drug chichin. Yakagaku Zasshi 87:1524–1528, 1967.
350. SAIKI Y, AKAHORI Y, MORINAGA K, ET AL. Gas chromatography of natural volatile oils. IV. Gas chromatography of the volatile oils of the plants belonging to the *Heterotropa* genus. 2. Yakagaku Zasshi 87:1539–1543, 1967.
351. SAIKI Y, AKAHORI Y, MORINAGA K, ET AL. Gas chromatography of natural volatile oils. V. Gas chromatography of the volatile oils of plants belonging to the *Heterotropa* genus. 3. Yakagaku Zasshi 87:1544–1547, 1967.

352. SAIKI Y, AKAHORI Y, and MORINAGA K. Gas chromatography of natural volatile oils. III. Gas chromatography of the volatile oils of plants belonging to the *Heterotropa* genus. 1. Yakagaku Zasshi 87:1535–1538, 1967.
353. MATTOCKS AR, SCHOENTAL R, CROWLEY HC, ET AL. Indicine: the major alkaloid of *Heliotropium indicum* L. J Chem Soc, 1961, pp 5400–5403.
354. MAN'KO IV. Alkaloids of *Cynoglossum officinale* and *Echium vulgare* and standard drug preparation from *Cynoglossum officinale*. Farm Zh 19:22–26, 1964.
355. MARQUEZ VC. Chromatographic separation of the alkaloids of *Bulnesis retamo*, *Heliotropium arborescens*, and *Cestrum auriculatum*. Bol Soc Quim Peru 27:161–172, 1961.
356. TRAUTNER EM, and NEUFELD OE. The alkaloids of *Heliotropium europeum* growing in Australia. Aust J Biol Sci 11:211–212, 1949.
357. CROWLEY HC, and CULVENOR CCJ. Alkaloid assays of *Heliotropium europaeum*. Aust J Appl Sci 7:359–364, 1956.
358. MEN'SHIKOV G. Alkaloids of *Heliotropium lasiocarpum* and *Trichodesma incanum* Fam. Boraginaceae. Bull Acad Sci URSS, Cl Sci Math Nat Ser Chim, 1936, pp 969–979.
359. MAN'KO IV, and VASIL'KOV PN. *Lappula intermedia* alkaloids. I. Tr Leningr Khim Farm Inst 26:166–173, 1968.
360. MAN'KO IV, MEL'KUMOVA ZV, and MALYAHAVA VF. Accumulation of alkaloids in different organs of *Symphytum caucasicum*. Rast Resur 8:538–541, 1972.
361. MURAKAMI T, and MATSUSHIMA A. Constituents of Japanese Berberidaceae plants. I. Constituent of the root of *Diphylleia grayi*. Yakagaku Zasshi 81:1596–1600, 1961.
362. SESHADRI TR, and SANKARA S. Components of Indian podophyllum. J Sci Ind Res 9B:137–141, 1950.
363. NADKARNI MV, MAURY PB, and HARTWELL JL. Components of podophyllin. VI. Isolation of two new compounds from *Podophyllum emodi*. J Am Chem Soc 75:280–281, 1952.
364. DE AMBROSI L. Rapid method of extraction of podophyllotoxin from the resin of *Podophyllum emodi*. Farmaco 9:265–267, 1954.
365. GRANADAS JR, and IRAZAZABAL NERPELL A. The active components of *Podophyllum emodi*. II. An R Soc Esp Fis Quim 50B:779–784, 1954.
366. CHAKRAVARTI SC, and CHAKRABORTY DP. Chemical examination of *Podophyllum emodi* var. *hexandrum*. J Am Pharm Assoc 43:614–615, 1954.
367. SPATH E, WESSELY F, and KORNFELD L. Constitution of podophyllotoxin and picropodophyllin. Chem Ber 65B:1536–1549. 1932.
368. VIEHOEVER A, and MACK H. Biochemistry of may-apple root (*Podophyllum peltatum*). I. J Am Pharm Assoc 27:632–643, 1938.
369. TSAREV MV. Chemical characteristics of *Podophyllum peltatum*. Farmatsiia 6:16–22, 1943.
370. HARTWELL JL. Alpha-peltatin, a new compound isolated from *Podophyllum peltatum*. J Am Chem Soc 69:2918, 1947.
371. PRESS J, and BRUN R. Some constituents of podophyllin. The structure of podophyllotoxin, alpha-peltatin, and beta-peltatin. Helv Chim Acta 37:190–202, 1954.
372. SCHRECKER AW, HARTWELL JL, and ALFORD WC. Components of podophyllin. XVIII. Polymorphic modifications of podophyllotoxin. J Org Chem 21:288–289, 1956.
373. ZOZULYA RN, KUZNETSOVA GA, MEL'NIKOVA TA, ET AL. Chemical and pharmacological studies of preparations isolated from *Podophyllum peltatum* growing in the Leningrad region. Tr Leningr Khim Farm Inst 13:245–252, 1961.
374. SHIBATA S, MURATA T, and FUJITA M. Constituents of Japanese and Chinese drugs. VI. Constituents of rhizome and roots of *Podophyllum pleianthum*. Yakagaku Zasshi 82:777–779, 1962.
375. MACHADO A. Chemical analyses of the seeds of *Protium brasiliensis*. Rev Bras Farm 25:155–156, 1944.
376. ADAMS R, and GIANTURCO M. *Senecio* alkaloids. The composition of hieracifoline and jacobine. J Am Chem Soc 78:398–400, 1956.
377. CULVENOR CCJ, and SMITH LW. The separation of *Senecio* alkaloids and the nature of hieracifoline and pterophine. Chem Ind, 1954, p 1386.
378. CULVENOR CCJ, and SMITH LW. The alkaloids of *Erechtites quadridentata*. Aust J Chem 8:556–561, 1955.
379. FERRY S. Alkaloids of some indigenous *Senecio* (species). I. Preliminary studies. Ann Pharm Fr 30:145–152, 1972.
380. WARREN FL. *Senecio alkaloids*. *In* Alkaloids (Manske RHF, ed). New York, Academic Press, 1970, vol 12, pp 245–331.
381. WARREN FL. The pyrrolizidine alkaloids. Prog Chem Org Nat Prod 12:198–269, 1955.
382. WILLAMAN JJ, and LI HL. Alkaloid-bearing plants and their contained alkaloids, 1957–1968. Lloydia 33:1–286, 1970.
383. WHITE EP. Alkaloids of some herbaceous *Senecio* species in New Zealand. N Zeal J Sci 12:165–170, 1969.
384. ALEKSEEV VS. Alkaloids of the 1-methylpyrrolizidine series (heliotridans). I. Alkaloids from some varieties of ragweed in the Ukraine and Sakhalin. Iz I Ispol'z Lekar Rast Resur SSSR Sb, 1964, pp 204–207.
385. ALEKSEEV VS. 1-Methylpyrrolizidine alkaloids. VII. Seneciphylline from *Senecio borystenicus*. Med Prom SSSR 15:27–29, 1961.
386. ADAMS R, and GIANTURCO M. *Senecio* alkaloids: the alkaloids of *Senecio brasiliensis*, *S. fremonti*, and *S. ambrosioides*. J Am Chem Soc 78:5315–5317, 1956.
387. MOTIDOME M, and FERREIRA PC. Alkaloids of *Senecio brasiliensis*. Rev Fac Farm Bioquim Univ Sao Paulo, 1966, pp 13–44.
388. ADAMS R, GIANTURCO M, and VAN DUUREN BL. Alkaloids from *Senecio tomentosus*. Observations on the alkaloid jacobine and on the structure of jaconecic acid. J Am Chem Soc 78:3513–3519, 1956.
389. WALI BK, and HANDA KL. Alkaloidal constituents of *Senecio chrysanthemoides*. Curr Sci 33:585, 1964.
390. WARREN FL. The pyrrolizidine alkaloids. II. Prog Chem Org Nat Prod 24:329–406, 1966.
391. ALEKSEEV VS, and BAN'KOVSKII OI. Pyrrolizidine alkaloids from some species of *Senecio*. Farm Zh 20:49–54, 1965.
392. HABIB AAM. Senecionine, seneciphylline, jacobine and otosenine from *Senecio cineraria*. Planta Med 26:279–282, 1974.
393. GHARBO SA, and HABIB AAM. Phytochemical investigation of Egyptian *Senecio*. II. Alkaloids of *Senecio aegyptius*, *S. desfontainei*, *S. vulgaris*, *S. petasitis* and *S. mikanioides*. Lloydia 32:503–508, 1969.
394. SCHOENTAL R. Alkaloidal constituents of *Senecio discolor*, retrorsine and isatidine. J Chem Soc, 1960, p 2375.
395. HENNIG AJ. *Senecio discolor*. The isolation and identification of senecionine and retrorsine. Lloydia 24:68–70, 1961.
396. BOTTOMLEY W, and GEISSMAN TA. Pyrrolizidine alkaloids. The biosynthesis of retronecine. Phytochemistry 3:357–360, 1964.
397. KOMPIS I, SCHROTER HB, POTESILOVA H, ET AL. Alkaloids of *Senecio erraticum barbaraeifolius*. II. Coll Czech Chem Commun 25:2449–2453, 1960.
398. KOMPIS I, and SANTAVY F. Alkaloids of *Senecio erucifolius*. Coll Czech Chem Commun 27:1413–1421, 1962.
399. BLACKIE JJ. The alkaloids of the genus *Senecio*. Pharm J 138:102–104, 1937.
400. LEONARD NJ. *Senecio* alkaloids. *In* Alkaloids (Manske RHF, and Holmes HL, eds). New York, Academic Press, 1950, Vol 1, pp 107–164.
401. GLONTI SHI. The process of alkaloid accumulation in *Senecio grandifolia* growing in a wild state in some regions of the Georgian SSR. Sb Tr Tbilis Nauch Issled Khim Farm Inst, 1958, pp 31–35.

402. MOTIDOME M, and FERREIRA PC. Alkaloids from the genus *Senecio*. Rev Fac Farm Bioquim Univ Sao Paulo 4:175–179, 1966.
403. DE WAAL HL. South African *Senecio* alkaloids. V. Notes on isatidine, rosmarinine and pterophine and on the structure of their necines and necic acids. Onderstepoort J Vet Sci Anim Ind 16:149–166, 1941.
404. DE WAAL HL. Toxic principles of *Senecio* species. The cause of dunsiekte in animals and bread poisoning in human beings. Farm S Afr 16:69–70, 1941.
405. DE WAAL HL. African *Senecio* alkaloids. Nature 146:777–778, 1940.
406. DE WAAL HL. The *Senecio* alkaloids. II. Hydrogenation, hydrolysis and structural results of isatidine. Onderstepoort J Vet Sci Anim Ind 14:433–447, 1940.
407. STEYN DG. Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa. VII. Onderstepoort J Vet Sci Anim Ind 9:111–124, 1937.
408. AKRAMOV ST, SHADMANOV Z, SAMATOV A, ET AL. Alkaloids of *Senecio jacobaea*, *Heliotropium acutiflorum*, and *H. transoxanum*. Khim Prir Soedin 4:258, 1966.
409. BRADBURY RB, and CULVENOR CCJ. The alkaloids of *Senecio jacobaea*. Chem Ind, 1954, pp 1021–1022.
410. BRADBURY RB, and MOSBAUER S. The alkaloids of *Senecio jacobaea*. Chem Ind, 1956, pp 1236–1237.
411. ALEKSEEV VS, and BAN'KOVSKII OI. Pyrrolizidine alkaloids from some species of *Senecio*. Farm Zh 20:49–54, 1965.
412. BRADBURY RB, and CULVENOR CCJ. The alkaloids of *Senecio jacobaea*. I. Isolation of the alkaloids and identification of jacobine as seneciphylline. Aust J Chem 7:378–383, 1954.
413. SAPUNOVA LA, and BAN'KOVSKII AI. Alkaloids of *Senecio krylovii*. Khim Prir Soedin 4:389, 1968.
414. KHALILOV DS, and TLEEZHENETSKAYA MV. Alkaloids of *Senecio*. Khim Prir Soedin 9:128–129, 1973.
415. KHALILOV DS, and DAMIROV IA. Alkaloid composition of *Senecio lampsanoides* from Azerbaidzhan flora. Farmatsiia 23:25–26, 1974.
416. DANILOV AV, KORETSKAYA NI, and UTKIN LM. A new alkaloid from *Senecio othonnae*. Zh Obshch Khim 32:647–648, 1962.
417. PRETORIUS TP. The alkaloids of *Senecio paucicalyculatus*. Onderstepoort J Vet Sci Anim Ind 22:297–300, 1949.
418. MURAV'EVA DA. The above-ground parts of *Senecio* as a source of platyphylline, sarracine, and seneciphylline alkaloids. Med Prom SSSR 19:49–52, 1965.
419. MURAV'EVA DA. *Senecio platyphyllus* and the alkaloid sarracine. Farm Zh 19:29–32, 1964.
420. DANILOVA AV, UTKIN LM, KOZYREVA GV, ET AL. A new alkaloid isomeric with platyphylline. Zh Obshch Khim 29:2432–2436, 1959.
421. MURAV'EVA DA. Platyphylline and sarracine and their sources. Med Prom SSSR 18:14–18, 1964.
422. KHALILOV DS, DAMIROV IA, and TELEZHENETSKAYA MV. Seneciphylline from *Senecio propinquus*. Khim Prir Soedin 5:656, 1972.
423. KHMEL MP. Alkaloids of *Senecio racemosus*. Farm Zh 16:35–39, 1961.
424. LEISEGANG EC. *Senecio* alkaloids. The ultraviolet extinction curves of the alkaloids and the necic acids. J S Afr Chem Inst 3:73–76, 1950.
425. MANSKE RHF. The alkaloids of *Senecio* species. III. *Senecio integerrimus*, *S. longilobus*, *S. spartioides* and *S. riddellii*. Can J Res B 17:1–7, 1939.
426. TRIVEDI B, and SANTAVY F. Alkaloids of the plant *Senecio subalpinus*. Coll Czech Chem Commun 28:3455, 1963.
427. GORDON-GRAY CG, and WELLS RB. Swazine, a new alkaloid from *Senecio swaziensis*. Chemical and x-ray crystal study of a novel spiro dilactone. Tetrahedron Lett, 1972, pp 707–710.
428. SHUN T, KOLUCH J, and SANTAVY F. Alkaloids of *Senecio vulgaris*. Coll Czech Chem Commun 25:934–939, 1960.
429. FERRY S. Alkaloids of some indigenous *Senecio* (species). I Preliminary studies. Ann Pharm Fr 30:145–152, 1972.
430. KERY A. Changes in the tertiary and *N*-oxide alkaloids of *Senecio vulgaris* by organs during the vegetative period. Herba Hung 12:29–36, 1973.
431. NIGAM SS, RAO A, and KAMESWARA A. Chemical study of essential oil from *Ocimum basilicum*. Riechst Aromen Koerperpflegem 18:169–170, 1968.
432. TSAI Y, TING CK, and NIEH SL. Essential oils of the family Lauraceae from Yunnan. I. Chemical constituents of the essential oils of *Cinnamomum glanduliferum* and *Cinnamomum bodinieri*. Yao Hsueh Hsueh Pao 11:801–808, 1964.
433. KIDDER MC, KATZ A, HALL AG, ET AL. Some potential sources of important plant products in California. Econ Bot 4:3–36, 1950.
434. FUJITA Y, FUJITA S, and TANAKA Y. Biogenesis of the essential oils in camphor trees. XXXIV. Essential oil components of a residue of *Cinnamomum camphora* excavated at Konoyama, Moriyama, Oe district in Tokushima Prefrecture, Japan. Nippon Nogei Kagaku Kaishi 48:693–696, 1974.
435. FUJITA Y, FUJITA S, and TANAKA Y. Biogenesis of the essential oils in camphor trees. XXXV. Components of an essential oil from the young trunk of *Cinnamomum camphora*. Nippon Nogei Kagaku Kaishi 48:697–700, 1974.
436. FUJITA Y, FUJITA S, and AKAMA Y. Biogenesis of the essential oils in camphor trees. XXIX. Comparison of the essential oils of the seedlings of *Cinnamomum camphora*. Cultivated in different daylight. Nippon Nogei Kagaku Kaishi 46:17–20, 1972.
437. NOGUCHI M, and KITAJIMA M. Seasonal changes of the essential oil content in camphor tree leaves. Nippon Ringaku Kaishi 41:488–494, 1959.
438. NAITO T. The constituents of the volatile oil from the leaf of *Cinnamomum camphora* var. *glaucescens*. J Chem Soc Jpn 64:1125–1129, 1943.
439. FUJITA Y, FUJITA S, and YOSHIKAWA H. Biogenesis of essential oils in camphor trees. XXXIII. Components of essential oils of *Cinnamomum doederleinii*. I. Nippon Nogei Kagaku Kaishi 48:633–636, 1974.
440. Massoia bark oil. Soap Perfum Cosmet 18:404, 1945.
441. IKEDA T. Essential oil of Pha-chium. J Chem Soc Jpn 51:335–348, 1930.
442. BROWN E, COOMES TJ, ISLIP HT, ET AL. Oils of *Cinnamomum* species from Sarawak as a source of safrole. Colon Plant Anim Prod 4:239–243, 1954.
443. LEAO DA SILVA M, MAIA JGS, ANDRADE DA, ET AL. Chemistry of Brazilian Lauraceae. XXIII. Arylpropanoids from *Licaria puchury-major*. Phytochemistry 12:471–472, 1973.
444. SEABRA AP, GUIMARAES EC, and MORS WB. Gas-liquid chromatography of "puxuri" essential oils. An Assoc Bras Quim 26:73–78, 1967.
445. FESTER GA, RETAMAR JA, and RICCIARDI AIA. Volatile materials from Argentine plants. Bol Acad Nac Cienc 42:13–22, 1960.
446. SHUKIS AJ, and WACHS H. Determination of safrole in the oil of *Ocotea cymbarum*. A cryoscopic method. Anal Chem 20:248–249, 1948.
447. MORS WB, MAGALHAES MT, and GOTTLIEB OR. Physiological varieties of *Ocotea pretiosa*. Perfum Essent Oil Rec 50:26–27, 1959.
448. MOLAN TRM. Essential oils of the sassafras laurels. II. The safrole-camphor type. Perfum Essent Oil Rec 52:349–350, 1961.
449. GEMBALLA G. Brazilian sassafras oil. Sci Pharm 26:8–14, 1958.
450. WALKER GT. Determination of safrole in sassafras oil. Seifen-Oele Fette-Wachse 94:451–453, 1968.

451. CLARK EP. The occurrence of rotenone and related compounds in the roots of *Cracca virginiana*. Science 77:311–312, 1933.

452. GANDHI RN, RAJAGOPALAN TR, and SESHARDI TR. Alkaloidal constituents of *Crotalaria grahamiana*. Curr Sci 35:514–515, 1966.

453. ATAL CK, CULVENOR CCJ, SAWHNEY RS, ET AL. Alkaloids of *Crotalaria grahamiana*. Grahamine, the 3'-[(-)-2-methylbutryl] ester of monocrotaline. Aust J Chem 22:1773–1777, 1969.

454. SURI OP, and ATAL CK. Isolation of monocrotaline and crispatine from *Crotalaria lechnaultii*. Curr Sci 36:614–615, 1967.

455. SAWHNEY RS, and ATAL CK. Phytochemical studies on the genus *Crotalaria*. VIII. Chemical constituents of *Crotalaria mysorensis*. J Indian Chem Soc 45:1052–1053, 1968.

456. CULVENOR CCJ, and SMITH LW. The alkaloids of *Crotalaria retusa*. Aust J Chem 10:464–473, 1957.

457. WILLETTE RE, and CAMMARATO LV. Phytochemical survey of Connecticut. I. Isolation of monocrotaline from *Crotalaria sagittalis* fruit. J Pharm Sci 61:122, 1972.

458. ATAL CK, and SAWHNEY RS. Pyrrolizidine alkaloids from Indian Crotalarias. Indian J Pharm 35:1–12, 1973.

459. KAY JM, and HEATH D. *Crotalaria spectabilis*. *In* The Pulmonary Hypertension Plant. Springfield, Ill, C. C. Thomas, 1969.

460. DEAN FM. Naturally Occurring Oxygen Ring Compounds. London, Butterworth, 1963, pp 485–548.

461. JONES MA, ET AL. Evaluation of some clones of *Derris elliptica*. Trop Agric 23:89–93, 1946.

462. HAMADA M, and CHUBACHI M. The origin of carbons of dihydrofuran ring and C-6 in the biosynthesis of rotenone. Agric Biol Chem 33:793, 1969.

463. KARIYONE T, KIMURA Y, and KONDO K. Constituents of *Derris* root. II. Yakagaku Zasshi 514:1049–1060, 1924.

464. TAKEI S. Rotenone, an active constituent of derris root (*Derris elliptica* Benth). Biochem Z 157:1–15, 1925.

465. LINCOLN R. Report of chemical division. *In* Mauritius Department of Agriculture Annual Report. 1936, pp 39–43.

466. GUNTHER FA, and TURRELL FM. Critical examination of the roots of *Derris elliptica*. J Econ Entomol 35:941, 1942.

467. EJERCITO JM. The rotenone content of Cebu derris. Philipp J Agric 10:187–194, 1939.

468. RAO NVS, and SESHADRI TR. Chemical examination of plant insecticides. I. Chemical components of *Derris ferruginea*. Proc Ind Sci 24A:344–348, 1946.

469. MOORE RH. Physiological studies of rotenone-bearing plants. *In* Puerto Rico Agricultural Experimental Station Annual Report, 1939, pp 71–93.

470. MARTIN JT, and TATTERSFIELD F. The problem of evaluation of rotenone-containing plants. II. *Derris elliptica*, *Derris malaccensis* and the "Sumatra-type" roots. Ann Appl Biol 23:880–898, 1936.

471. SCHUITEMAKER JP. Occurrence and properties of certain varieties of tuba in West Borneo. Ind Mer 58:165–168, 1935.

472. WORSLEY RRLG. Report on biochemistry. *In* East African Agricultural Research Station Amani, Annual Report, 1938, pp 27–31.

473. CAMINHA A, JR. Rotenone content of some Brazilian species. Ind Quim 1:171–172, 1936.

474. SAVAGE N, and GROENEWOUD PWG. South African fish-poisonous plants. III. Preliminary chemical investigation of the tuber of *Neorautanenia ficifolia*. J S Afr Chem Inst 7:1–10, 1954.

475. HWANG SL. A preliminary report on the chemical composition of yam bean (*Pachyrhizus erosus* Urban), a new rotenone-bearing plant. Kwangzi Agric 2:269–280, 1941.

476. RICHMOND DV, and MARTIN JT. Determination of rotenone in *Lonchocarpus* root and oleoresin. J Sci Food Agric 10:404–408, 1959.

477. CLARK EP. Occurrence of rotenone and related substances in the seeds of the berebera tree. A procedure for the separation of deugelin and tephrosin. J Am Chem Soc 65:27–29, 1943.

478. GHOSE TP, and KRISHNA S. Occurrence of rotenone in *Millettia pachycarpa*. Curr Sci 6:57, 1973.

479. KARIYONE T, ATSUMI K, and SHIMADA M. The constituents of *Millettia taiwaniana* Hayata. Yakagaku Zasshi 500:739–746, 1923.

480. FALSHAW CP, OLLIS WD, MOORE JA, ET AL. The extractives of *Piscidia erythrina*. III. The constitutions of lisetin, piscidone, and piscerythrone. Tetrahedron 7(suppl):333–348, 1966.

481. KRISHNA S, and GHOSE TP. *Tephrosia* sp. as a source of rotenone. Curr Sci 6:454, 1938.

482. CHEN YL, and HSU HY. Constituents of *Tephrosia* species in Taiwan. I. Active principle of *Tephrosia obovata*. Yakagaku Zasshi 78:198–200, 1958.

483. HOS O, and JONES A. Colorimetric evaluation of *Derris* and cube roots. *In* Industrial Engineering and Chemical Analysis, 11th ed, 1939, p 429.

484. GINSBURG JM, ET AL. Rotenone-bearing variety of *Tephrosia virginiana* in New Jersey. J Econ Entomol 35:276–280, 1942.

485. LITTLE VA. Rotenone content, an inherited character in the roots of Devil's shoestring, *Tephrosia virginiana*. J Econ Entomol 35:54–57, 1942.

486. GOODHUE LD, and HALLER HL. The non-crystalline constituents of *Tephrosia virginiana* roots. J Am Chem Soc 62:2520–2522, 1940.

487. JONES HA, and SULLIVAN WN. Chemical and insecticidal tests of samples of *Tephrosia toxicaria*. J Econ Entomol 30:679–680, 1937.

488. WEISS SG, TIN-WA M, PERDUE RE, JR, ET AL. Potential anticancer agents. II. Antitumor and cytotoxic lignans from *Linum album* (Linaceae). J Pharm Sci 64:95–98, 1975.

489. ALTMAN RFA. Chemical studies of Amazonian plants. I. Microchemical identification of *Cinchoma* alkaloids. Bol Tec Inst Agrom Norte (Belem, Brazil) 31: 9–66, 1956 (abstracted in Chem Abstr 52:506a, 1960).

490. NAGASAWA M, MURAKAMI T, IKEDA K, ET AL. Geographical variation of essential oils of flos magnoliae. Yakagaku Zasshi 89:454–459, 1969.

491. MONTE AL. Essential oils of aromatic plants from Nahuel Huapi National Park and its adjacent regions. V. Essential oils of *Myrceugenia exsucca* and *Laurelia serrata*. An Soc Cient Argent 178:110–124, 1964.

492. GONZALEZ ME, and MONTES AL. Essential oil of *Laurelia serrata* from *Laguna friaseugen*. An Asoc Quim Argent 42:152–166, 1954.

493. FESTER GA, MARTINUZZI EA, and RICCIARDI AI. Volatile oils. IV. Rev Fac Ing Quim 20:47–60, 1951.

494. BEJNAROWICZ EA, and KIRCH ER. Gas chromatographic analysis of oil of nutmeg. J Pharm Sci 52:988–993, 1963.

495. ITTY MI, and NIGAM SS. Essential oil of *Myristica fragrans*. Reichst Aromen Koerperpflem 16:399–400, 1966.

496. SHULGIN AT, SARGENT T, and NARANJO C. The chemistry and psychopharmacology of nutmeg and of several related phenylisopropylamines. Washington, DC, US Public Health Service Publication No. 1645. 1967, pp 202–214.

497. SLAVIKOVA L, SHUN T, and SLAVIK J. Alkaloids of Papaveraceae. XIV. Alkaloids of *Argemone alba*. Coll Czech Chem Commun 25:756–760, 1960.

498. KABELIK J. Sanguinarine (as the cause of) glaucoma. Cesk Oftalmol 22:49–52, 1966.

499. SLAVIKOVA L, and SLAVIK J. Alkaloids of Papaveraceae. VII. *Argemone mexicana*. Chem Listy 49:1546–1549, 1955.

500. BUI TY. A chemical study of the seeds of *Argemone mexicana* L. cultivated in the USSR and indigenous to Vietnam. Farmatsiia 23:36–38, 1974.
501. BORI TY, and MURAV'EVA DA. Isolation and determination of the alkaloids of *Argemone mexicana* grown in different geographic regions. Rast Resur 9:200–202, 1973.
502. SLAVIK J, and SLAVIKOVA L. Alkaloids of the Papaveraceae. XII. Alkaloids of *Argemone platyceras*. Coll Czech Chem Commun 28:1728–1737, 1963.
503. HAISOVA K, and SLAVIK J. Alkaloids of the Papaveraceae. LV. Alkaloids from *Argemone polyanthemos*. Coll Czech Chem Commun 39:2491–2493, 1974.
504. BANDONI AL, RONDINA RVD, and COUSSIO JD. Alkaloids of Argentina medicinal plants. III. Alkaloids of *Argemone subfusiformis*. Phytochemistry 11:3547–3548, 1972.
505. KIRYAKOV NG, KITOVA MS, and GEORGIEVA AV. Alkaloids of *Bocconia cordata*. C R Acad Bulg Sci 20:189–192, 1967.
506. DOMINGUEZ XA, DELGADO JG, MONROY CA, ET AL. A chemical study of *Bocconia latisepala*. Can J Chem 43:679–682, 1965.
507. GRABARCZYK H, and GERTIG H. Chromatography of tertiary and quaternary alkaloids occurring in the above-ground organs of *Bocconia microcarpa*. Ann Pharm 8:75–81, 1970.
508. CHELOMBIT'KO VA, and MURAV'EVA DA. *Bocconia microcarpa* source of alkaloids chelerythrine and sanguinarine. Khim Farm Zh 2:49–52, 1968.
509. GRABARCZYK H, and GERTIG H. Tertiary and quaternary alkaloids occurring in the roots of *Bocconia microcarpa*. Ann Pharm 7:105–110, 1969.
510. LABRIOLA RA, and GIACOPELLO D. Alkaloids of the stem and root of *Bocconia pearcei*. An Asoc Quim Argent 54:1527, 1966.
511. JUSIAK L. Separation of *Chelidonium majus* alkaloids by countercurrent cascade extraction. II. Acta Pol Pharm 24:65–70, 1967.
512. SLAVIK J. Alkaloids of Papaveraceae. XIII. Alkaloids from *Glaucium vitellinum*. Coll Czech Chem Commun 24:3999–4003, 1959.
513. YAKHONTOVA LD, TOLKACHEV ON, and KUBAL'CHICH PN. *Chelidonium majus*, a raw material for producing the drug sanguiritrin. Farmatsiia 22:31–33, 1973.
514. GULUBOV A, SUNGURYAN T, BOZHKOVA H, ET AL. *Chelidonium majus* alkaloids. I. Nauch Tr Vissh Pedagog Inst Plovdiv Mat Fiz Khim Biol 6:63–65, 1968.
515. LAVENIR R, and PARIS RR. Alkaloids of *Chelidonium majus*. Distribution in various tissues: isolation of stylopine from the fruits. Ann Pharm Fr 23:307–312, 1965.
516. PLATONOVA TF, MASSAGETOV PS, KUZOVKOV AD, ET AL. Alkaloids of plants of the poppy family. Zh Obshch Khim 26:173–180, 1956.
517. SLAVIK J, SLAVIKOVA L, and BRABENEC J. Alkaloids of Papaveraceae. XXX. Other alkaloids from the roots of *Chelidonium majus*. Coll Czech Chem Commun 30:3697–3704, 1965.
518. KRATZMANN E. Microscopical detection of *Chelidonium* alkaloids. Pharm Monatsh 3:45–50, 57–66, 1922.
519. KRATZMANN E. Latex of *Chelidonium majus*. II. Distribution of alkaloids in the plant. Pharm Monatsh 5:161–164, 1924.
520. KRATZMANN E. The alkaloids of *Chelidonium majus* L. Mikrokosmos 1:176–179, 1924.
521. GHEORGHIU A, CONSTANTINESCU A, and IONESCU-MATIU E. Separation and identification of fluorescent alkaloids of the pharmaceutical forms of *Chelidonium majus* by electrophoresis. Acad Rep Pop Romtne Inst Biochim Studii Cercetdri Biochim 2:271–277, 1959.
522. KAJDEROWICZ-JAROSINSKA D. Differentiation of preparations of *Chelidonium majus* by disk paper chromatography. Dissert Pharm 13:357–361, 1961.
523. GHEORGHIU A, IONESCU-MATIU E, and LUPULESCU V. Determination of total alkaloids and evaluation of the percent content of some alkaloids in *Chelidonium majus* collected from natural basins of Romania. Stud Cercet Biochim 15:161–166, 1972.
524. JANS BP. Studies on the milky sap of *Chelidonium majus* L. (Papaveraceae). Bull Soc Bot Suisse 83:306–344, 1974.
525. TANI C, and TAKAO N. Alkaloids of Fumaricaceae. I. Alkaloids of *Corydalis incisa*. I. Tertiary alkaloids. Yakagaku Zasshi 82:594–598, 1962.
526. NIKOL'SKAYA BS. Pharmacology of sanguinarine, an anticholinesterase alkaloid. Farmakol Toksikol 29:76–79, 1966.
527. SHEVELEVA GP, SARGAZAKOV D, PLEKHANOVA NV, ET AL. Alkaloids of *Veratrum lobelianum*, *Fumaria vaillantii*, and *Corydalis ledebouriana*. Kirgiz flora. Fiziol Aktiv Soedin Rast Kirg, 1970, pp 41–56.
528. MARGVELASHVILI NN, and PAKALN DA. Composition of alkaloids of *Corydalis persica*. Khim Prir Soedin 9:133, 1973.
529. BAISHEVA KH SH, and ROSTOTSKII BK. *Corydalis stricta* and *Corydalis fimbrillifera* alkaloids. Lek Rast 15:376–381, 1969.
530. MARGVELASHVILI NN, LASSKAYA OE, KIR'YANOVA AT, ET AL. Alkaloids of *Corydalis vaginans*. Khim Prir Soedin 7:813, 1974.
531. SLAVIK J. Alkaloids of the Papaveraceae. X. Secondary alkaloids from *Papaver rhoeas* and *Dicentra spectabilis*. Chem Listy 52:1957–1964, 1958.
532. SLAVIKOVA L. Alkaloids of Papaveraceae. XXXIV. Alkaloids from *Glaucium squamigerum*. Coll Czech Chem Commun 31:4181–4184, 1966.
533. SLAVIKOVA L, and SLAVIK J. Alkaloids of Papaveraceae. XXXII. Alkaloids of *Hunnemannia fumariaefolia* and constitution of the alkaloid HF 1. Coll Czech Chem Commun 31:1355–1362, 1966.
534. SLAVIK J, and SLAVIKOVA L. Alkaloids of the Papaveraceae. XIX. Alkaloids from *Hypecoum procumbens* and *H. leptocarpum*. Coll Czech Chem Commun 26:1472–1475, 1961.
535. CHELOMBIT'KO VA. Experimental cultivation of *Macleaya microcarpa* under Caucasian mineral water conditions. Rast Resur 7:585–587, 1971.
536. MURAV'EVA DA, and CHELOMBIT'KO VA. Variations in the amounts of chelerythrine and sanguinarine alkaloids in *Macleaya microcarpa* during growth. Arh Farm 19:1–5, 1969.
537. MURAV'EVA DA, and CHELOMBIT'KO VA. Seasonal variation of chelerythrine and sanguinarine alkaloids in *Macleaya microcarpa*. Acta Pharm Jugoslav 19:11–14, 1969.
538. SLAVIK J, and SLAVIKOVA L. Alkaloids of Papaveraceae. VI. Alkaloids of *Macleaya microcarpa*. Chem Listy 49:106–110, 1955.
539. GERTIG H. Chromatography of tertiary and quaternary alkaloids occurring in the roots of *Meconopsis rudis*. Ann Pharm 7:111–115, 1969.
540. PFEIFER S, and KUEHN L. Papaver alkaloids. XXVI. *Papaver persicum*, *P. triniaefolium*, *P. fugax* and *P. polychaetum*. 2. Phenolic bases. Pharmazie 23:267–281, 1968.
541. PFEIFER S. Glaudine, new *Papaver* alkaloid. Pharmazie 19:724, 1964.
542. KUEHN L, and PFEIFER S. Alkaloids of the genus *Papaver*. VIII. Pharmazie 20:520–521, 1965.
543. BOEHM H. Characterization of alkaloid spectra of northern poppies. Abh Deut Akad Wiss Berlin Kl Chem Geol Biol 3:825–828, 1966.
544. SLAVIK J, and APPELT J. Alkaloids of Papaveraceae. XXIX. Alkaloids of some *Papaver* species. Coll Czech Chem Commun 30:3687–3696, 1965.
545. SLAVIK J, and SLAVIKOVA L. Alkaloids of the Papaveraceae. XXIV. Alkaloids of *Platystemon californicus*. Coll Czech Chem Commun 28:1917–1919, 1963.
546. SCHENCK G, and HANNSE H. The separation of sanguinarine

and chelerythrine and the associated discovery of new alkaloids from *Sanguinaria canadensis*. Arch Pharm 287:544–548, 1954.

547. TIN-WA M, FONG HHS, ABRAHAM DJ, ET AL. Structure of sanguidimerine, a new major alkaloid from *Sanguinaria canadensis* (Papaveraceae). J Pharm Sci 61:1846–1847, 1972.
548. SLAVIK J. Alkaloids of the Papaveraceae. XVII. New alkaloids from *Sanguinaria canadensis*. Coll Czech Chem Commun 25:1667–1675, 1960.
549. SLAVIK J. Alkaloids of the Papaveraceae. XX. Alkaloids of *Stylophorum diphyllum*. Coll Czech Chem Commun 26:2933–2939, 1961.
550. RUSSELL GF, and JENNINGS WG. Constituents of black pepper. Oxygenated compounds. J Agric Food Chem 17:1107–1112, 1969.
551. WASICKY R, UNTI O, and BARBIERI E. Quinine and alkaloids in Brazil. An Fac Farm Odontol Univ Sao Paulo 3:137–183, 1942–1943.
552. VAN LEERSUM P. The occurrence of quinine in the seeds of *Cinchona ledgeriana*, Moens. Pharm Weekbl 50:1464–1467, 1915.
553. WASICKY R. The problem of quinine and its alkaloids in Brazil. An Asoc Quim Bras 3:44–60, 1944.
554. DUTTA NL, and QUASSIM C. Isolation and characterization of a glycoside and alkaloids from heartwood of *Cinchona ledgeriana*. Indian J Chem 6:566–567, 1968.
555. HODGE WH. Reminiscences of a *Cinchona* hunter. Nat Hist 57:8–15, 41, 1948.
556. HODGE WH. Alkaloid distribution in the bark of some Peruvian cinchonas. Caribbean Forest 7:79–86, 1946.
557. MORRISON FR, PENFOLD AR, and SIMONSEN J. Essential oils of *Zieria smithii* and its various forms. II. J Proc R Soc N S Wales 84:196–201, 1950.
558. BOITEAU P, BUZAS A, LEDERER E, ET AL. Chemical constitution of asiaticoside, a heteroside formerly used against leprosy. Bull Soc Chim Biol 31:46–51, 1949.
559. BOITEAU P, and RATSIMAMANGA AR. Asiaticosid extracted from *Centella asiatica*, its therapeutic uses in the healing of experimental or refractory wounds, leprosy, skin tuberculosis, and lupus. Therapie 11:125–149, 1956.
560. BOITEAU P, and RATSIMAMANGA AR. Cicatrizants of vegetable origin and the biostimulins of Filatov. Bull Soc Sci Bretagne 32:28, 1957.
561. RAHANDRAHA T, CHANEZ M, and BOITEAU P. Determination of the anthrone asiaticoside, an oside ester in *Centella asiatica*. Ann Pharm Fr 21:313–320, 1963.
562. PASICH B, KOWALEWSKI Z, and SOCHA A. The triterpenoid and sterol compounds in plant material. XIII. The isolation of asiaticoside from the herb *Centella asiatica* using cationite. Diss Pharm Pharmacol 20:69–72, 1968.
563. SINGH B, and RASTOGI RP. Examination of *Centella asiatica*. IV. Reinvestigation of the triterpenes of *Centella asiatica*. Phytochemistry 8:917–921, 1969.
564. AUGUSTINE DL. The therapeutic value of two new sulfonamide compounds, succinylsulfathiazole and phthaloylsulfathiazole, in experimental trichinosis. N Engl J Med 230:349–350, 1944.
565. LAERUM O, and IVERSEN OH. Reticuloses and epidermal tumors in hairless mice after topical skin applications of cantharidin and asiaticoside. Cancer Res 32:1463–1469, 1972.
566. BERENBLUM I. The cocarcinogenic action of croton resin. Cancer Res 1:44–48, 1941.
567. BERENBLUM I. The mechanism of carcinogenesis. A study of the significance of cocarcinogenic action and related phenomena. Cancer Res 1:807–814, 1941.
568. HECKER E, BRESCH H, and VON SZCZEPANSKI CH. Cocarcinogen A-1—the first pure, highly active constituent of croton oil. Angew Chem 3:227, 1964.
569. HECKER E. New phorbol esters and related cocarcinogens. *In* Proceedings of the 10th International Cancer Congress, 1971, vol 5, pp 213–224.
570. ARMUTH V, and BERENBLUM I. Systemic promoting action of phorbol in liver and lung cocarcinogenesis in AKR mice. Cancer Res 32:2259–2262, 1972.
571. BAIRD WM, SEDGWICK JA, and BOUTWELL RK. Effects of phorbol and four diesters of phorbol on the incorporation of tritiated precursors into DNA, RNA, and protein in mouse epidermis. Cancer Res 31:1434–1439, 1971.
572. RAICK AN. Ultrastructural, histological, and biochemical alterations produced by 12-*O*-tetradecanoyl-phorbol-13-acetate on mouse epidermis and their relevance to skin tumor promotion. Cancer Res 33:269–286, 1973.
573. CHOUROULINKOV I, and LAZAR P. Actions cancerogenie et cocancerogene du 12-*O*-tetradecanoyl-phorbol-13-acetate. C R Acad Sci[D] 278:3027–3030, 1974.
574. RAICK AN. Ultrastructural, histological, and biochemical alterations produced by 12-*O*-tetradecanoyl-phorbol-13-acetate on mouse epidermis and their relevance to skin tumor promotion. Cancer Res 33:269–286, 1973.
575. VAN DUUREN BL, SIVAK A, SEGAL A, ET AL. Dose-response studies with a pure tumor-promoting agent, phorbol myristate acetate. Cancer Res 33:2166–2172, 1973.
576. WHITFIELD JF, MACMANUS JP, and GILLAN DJ. Calcium-dependent stimulation by a phorbol ester (PMA) of thymic lymphoblast DNA synthesis and proliferation. J Cell Physiol 82:151–156, 1973.
577. VAN DUUREN BL, BLAZEJ T, GOLDSCHMIDT BM, ET AL. Cocarcinogenesis studies on mouse skin and inhibition of tumor induction. J Natl Cancer Inst 46:1039–1044, 1971.
578. GSCHWENDT M, and HECKER E. Über die Wirkstoffe der Euphorbiaceen. I. Hautreizende und cocarcinogene Faktoren aus *Euphorbia cooperi*. J Natl Cancer Inst 80:335–350, 1973.
579. HERGENHAHN M, KUSUMOTO S, and HECKER E. Diterpene esters from "Euphorbium" and their irritant and cocarcinogenic activity. Experientia 30:1438–1440, 1974.
580. HERGENHAHN M, ADOLF W, and HECKER E. Resiniferatoxin and other esters of novel polyfunctional diterpenes from *Euphorbia resinifera* and *unispina*. Tetrahedron Lett, 1975, pp 1595–1998.
581. GSCHWENDT M, and HECKER E. Über die Wirkstoffe der Euphorbiaceen. II. Hautreizende und cocarcinogene Faktoren aus *Euphorbia triangularis* Desf. Z Krebs-forsch Klin Onkol 81:193–210, 1974.
582. KINGHORN AD, and EVANS FJ. Skin irritants of *Euphorbia fortissima*. J Pharm Pharmacol 27:329–333, 1975.
583. UEMURA D, OHWAKI H, and HIRATA Y. Isolation and structures of 20-deoxyingenol new diterpene, derivatives and ingenol derivative obtained from "Kansui." Tetrahedron Lett, 1974, pp 2527–2528.
584. UEMURA D, and HIRATA Y. New diterpene, 13-oxyingenol, derivative isolated from *Euphorbia kansui* Liou. Tetrahedron Lett, 1974, pp 2529–2532.
585. OPFERKUCH HJ, and HECKER E. New diterpenoid irritants from *Euphorbia ingens*. Tetrahedron Lett, 1974, pp 261–264.
586. ADOLF W, and HECKER E. On the irritant and cocarcinogenic principles of *Hippomane mancinella*. Tetrahedron Lett, 1975, pp 1587–1590.
587. SAKATA K, KAWAZU K, and MITSUI T. Piscicidal constituent of *Hura crepitans*. I. Isolation and characterization of huratoxin and its piscicidal activity. Agric Biol Chem 35:1084–1091, 1971.
588. OKUDA T, YOSHIDA T, KOIKE S, ET AL. New diterpene esters from *Aleurites fordii* fruits. Phytochemistry 14:509–515, 1975.
589. SCHILDKNECHT H, EDELMANN G, and MAURER R. Protective plant material. VII. Chemistry of mezerein, the inflamma-

tory and carcinogenic poison from *Daphne mezereum*. Chem Ztg Chem App 94:347–355, 1970.

590. SCHILDKNECHT H, and MAURER R. Protective plant material. VIII. Structure of mezerein from the fruit of the mezereon, *Daphne mezereum*. Chem Ztg Chem App 94:849, 1970.

591. RONLAN A, and WICKBERG B. The structure of mezerein, a major toxic principle of *Daphne mezereum* L. Tetrahedron Lett, 1970, pp 4261–4264.

592. STOUT GH, BALKENHOL WJ, POLING M, ET AL. Isolation and structure of daphnetoxin, the poisonous principle of *Daphne* species. J Am Chem Soc 92:1070–1071, 1970.

593. KUPCHAN SM, and BAXTER RL. Mezerein: antileukemic principle isolated from *Daphne mezereum* L. Science 187:652–653, 1975.

594. ROE FJC. Oil of sweet orange: a possible role in carcinogenesis. Br J Cancer 13:92–93, 1959.

595. ROE FJC, and PEIRCE WEH. Tumor promotion by citrus oils: tumors of the skin and urethral orifice in mice. J Natl Cancer Inst 24:1389–1402, 1960.

596. PEIRCE WEH. Tumour-promotion by lime oil in the mouse forestomach. Nature 189:497–498, 1961.

597. FIELD WEH, and ROE FJC. Tumor promotion in the forestomach epithelium of mice by oral administration of citrus oils. J Natl Cancer Inst 35:771–784, 1965.

598. SLAVIK J, and SLAVIKOVA L. Alkaloids of the Papaveraceae. XI. Further alkaloids of *Glaucium flavum* Cr. and *Glaucium flavum* var. *fulvum* (Smith) Fedde. Coll Czech Chem Commun 24:3141–3147, 1959.

599. IVANOVA V, and IVANOVA L. Alkaloid content of *Glaucium flavum* var. *lejocarpum*. Farmatsiia 8:27–33, 1958.

ANNOUNCEMENTS

Fellowship in Medical Oncology

Applications are now being accepted from candidates who are interested in a Joint Collaborative Training Program in Medical Oncology involving major teaching hospitals of Brown University's Program in Medicine, beginning July 1977 and July 1978. This program offers clinical and research activities in clinical pharmacology and cancer chemotherapy, biochemical pharmacology, drug development, and tumor immunology; it also participates in national cooperative chemotherapy groups to aid in the treatment of patients with solid tumors and hematologic malignancies which are seen at many of the hospitals in the Providence, Rhode Island, area.

Prerequisites include 2 years of approved medical residency and ABIM eligibility. Candidates with academic interests are encouraged to apply. *Send a letter and curriculum vitae to:* Paul Calabresi, MD, Professor and Chairman, Department of Medicine, or F. J. Cummings, MD, Head, Division of Medical Oncology, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Ave, Providence, RI 02908.

Third Annual Meeting of the Association of Community Cancer Centers

Rosslyn, Virginia, January 28–30, 1977

The Association of Community Cancer Centers (ACCC) announces its Third Annual Meeting to be held on Friday, January 28, to Sunday, January 30, 1977, at the Key Bridge Marriott Hotel in Rosslyn, Virginia. The ACCC is an organization composed of physicians and other health care providers dedicated to improving the quality of cancer care and treatment at the community level.

The meeting topics will be: (*a*) Psychosocial Considerations of Cancer Care; (*b*) Allied Health Personnel and the Team Concept of Cancer Care; and (*c*) Developing a Community Cancer Program. In addition, workshops will be conducted on various areas of interest to community health professionals.

The meeting is open to physicians, hospital administrators, nurses, rehabilitation specialists, and others who are interested in community cancer programs. Advance registration is recommended; the fee will be $50.00 for non-physician members, $75.00 for physician members, and $100.00 for non-members. *For further information please write:* ACCC 1977 Meeting, PO Box 30279, Bethesda, Md 20014.

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